GENETICS

A periodical record of investigations bearing on heredity and variation

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VOLUME 44 SEPTEMBER, 1959 NO. 5, PART 1

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EFFECT OF ENVIRONMENT UPON THE MANIFESTATION OF HETEROSIS AND HOMEOSTASIS IN DROSOPHILA PSEUDOOBSCURA

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A NUMBER of studies involving the comparison of the F_1 and F_2 generations from crosses between geographic populations of Drosophila with the populations themselves have been carried out. In general the findings were that F_1 hybrids showed a heterotic increase in Darwinian fitness over the parental populations and the F_2 generation showed a breakdown of heterosis.

Possible explanations for this kind of effect are of some interest and have been discussed by several workers, for example, by WALLACE and VETUKHIV (1955). However, the present experiments were initiated with another end in view. A number of observations had shown that the relative performance of two or more genotypes may be different under different environmental conditions. It appeared interesting to test whether the heterosis and breakdown observed in crosses between geographic populations at 25°C was maintained over a range of environmental conditions. Experiments of BEARDMORE (unpublished data) had shown that the superiority in developmental stability of F_1 hybrids over their inbred parents was dependent upon the type of environment in which the experiments were carried out. In an environment embodying a regularly fluctuating temperature cycle, Drosophila F_1 hybrids were not significantly more stable or homeostatic than the inbred parental lines, although under constant temperature conditions the F_1 superiority was consistently manifested as a lower degree of asymmetry of the sternopleural chaetae.

In the present study we tested whether F_1s derived from crosses between geographic populations show heterosis and whether the F_2s show breakdown over a range of environmental conditions. Comparable experiments testing flies homozygous and heterozygous for various second chromosomes of *D. pseudoobscura* have been described by DOBZHANSKY and WALLACE (1953). These experiments were concerned essentially with the between-homologues component of heterosis in F_1s, whereas in the present study we have looked at the over-all interaction between haploid sets of chromosomes in the F_1 and, as well, at recombination between sets in the F_2 generation.

MATERIALS AND METHODS

The material for the experiments consisted of 16 strains of *D. pseudoobscura* derived from wild flies collected at Gunnison, Colorado, and of ten strains from

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Mather, California. Each strain is descended from a single impregnated wild female. All the strains were homozygous for the Arrowhead gene arrangement in the third chromosomes. The strains were the same as those used in experiments reported previously by VETUKHIV (1953, 1956, 1957).

Virgin females were taken from one half of the strains and placed together with males from the other half of the strains. Reciprocal crosses were made using males from the first half of the strains and the females from the second half. Mather × Gunnison F_1 and F_2 hybrids were produced, and reciprocally, Gunnison × Mather F_1 and F_2 generations. The components of Darwinian fitness used to compare the three generations of flies were, larval viability (to eclosion), fecundity, (i.e., egg production) and the quantitative asymmetry of the wings. The larval viability was measured by putting 20 small first instar larvae within each of a number of vials for each genotype. The food used was KALMUS (1943) synthetic medium with a controlled amount of dried inactive yeast added.

For the fecundity studies, five females and five males were placed in each vial. Since five replicates were made, 25 females and 25 males were taken for each cross. Thus 50 females of the parental forms were taken for egg laying in each experiment, 50 females of F_1s and 50 of F_2s. Paper spoons with cream-of-wheat and molasses medium with a drop of freshly made suspension of Fleischmann's yeast were changed daily in each vial and the eggs were counted.

The wing asymmetry, that is the difference in length between right and left wings regardless of sign, was determined by detaching and measuring with an eyepiece micrometer both wings from each of 50 flies of each sex for each genotype.

The three environments used were, 25°C, the temperature at which the previous work had been carried out, 22°C, and a fluctuating temperature condition (designated F°) which changed from 17°C to 27°C and back each 24 hours.

The viability data

The data presented in histogram form in Figure 1 show for each of the six genotypes, the mean number of flies hatching per 20 larvae. The mean is derived from 15 replicate vials per genotype.

The data are split into three groups by environment. At 25° the F_1s show significantly greater survival than the parental forms and the tendency to break down is observed in F_2s against F_1s. In comparison with the P population, F_2 occupies an intermediate position, it is greater than one of the parents and less than the other one. In the 22°C and F° environments no significant differences are observed between genotypes, with the exception that in F°, F_2 is apparently superior to F_1.

The variability of survival of larvae (that is, the variance in sets of replicate vials) is shown in Table 1. It is readily seen that at 25°C, and only at that temperature, the parental genotypes show greater variance than the F_1s, as do the F_2s. This variance is in all cases of both genetic and environmental origin but the genetic variance will be expected to be equal in F_1 and in P generations; hence a

lower phenotypic variance is indicative of a superior homeostasis in the development of the F_1 phenotypes. In the F_2, of course, the fact that the variance is significantly greater than in F_1 could mean that there has been a breakdown of buffering capacity, that is, an increased sensitivity to environmental disturbance and inequalities. We are unable, however, to distinguish between the two com-

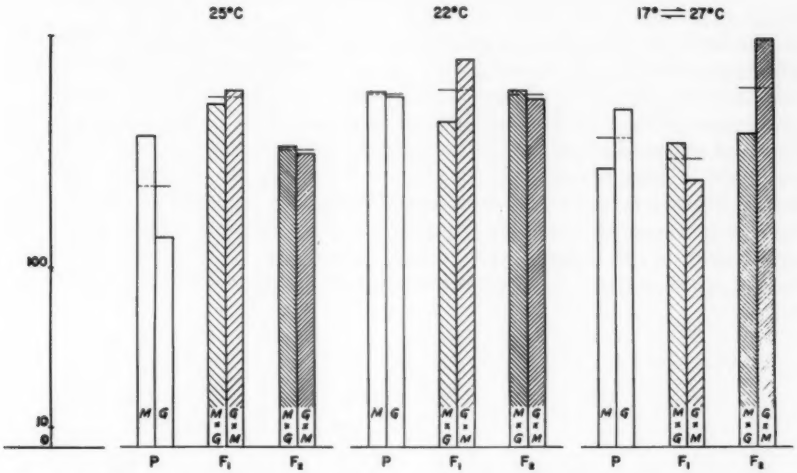

FIGURE 1.—Mean number of flies hatching per 20 larvae. M denotes the flies from Mather, California. G denotes the flies from Gunnison, Colorado. The horizontal line across two bars shows the mean between the values presented by each of the bars.

TABLE 1

Comparative variation in survival of larvae in replicate cultures

VARIANCE RATIO	P/F1		F2/F1		F2/P	
	min	max	min	max	min	max
25^0	2.7+	6.0++	2.7+	5.0++	0.6	1.9
22^0	0.5	2.1	1.2	0.5	0.48	1.0
f^0	0.6	0.7	0.65	0.78	0.95	1.2

min = smallest variance ratio of four possible.
max = largest variance ratio of four possible.
+ P = <0.05.
++ P = <0.01.
$f^o = F^o$.

ponents of variance, genetic and environmental; and recombination and segregation should in F_2 give rise to a generation with a genetic variance greater than in F_1 and P generations.

Note that in both of the other environments no significant differences are evident.

The fecundity data

The fecundity data are seen in Figures 2 and 3. Figure 2 shows the mean lifetime egg production per female, Figure 3, the mean daily egg production. As with the viability figures, the data are divided according to the test environment.

To consider first the data in Figure 2, it is clear that the F_1s are heterotic in terms of egg production at 25°C, and the F_2 generation is less fecund than P, though this latter difference is statistically not significant. In both of the other environments no statistically significant heterosis or breakdown was detected. This picture is exactly paralleled by that shown by Figure 3—the mean lifetime egg production—F_1 heterosis at 25°C, and no significant breakdown in F_2; at 22°C and F° 17/27 no statistically significant heterosis or breakdown observed in any case.

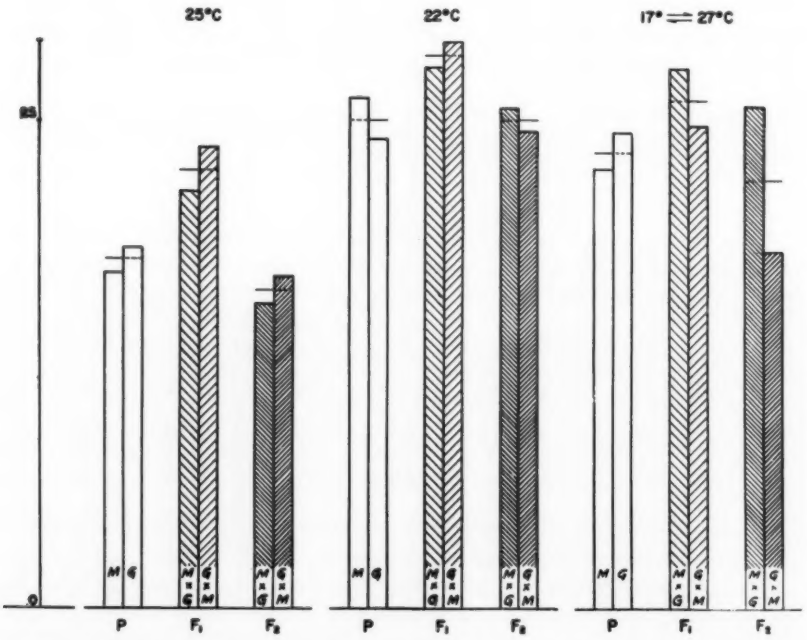

FIGURE 2.—Mean daily egg production per female.

The asymmetry data

The experimental results are shown in Figure 4 which expresses the quantitative difference between the two wings of individuals as the mean difference in micrometer units. One such unit is equivalent to about 1/800 total distance between the base of a bristle just anterior to the humeral cross vein and the tip of the wing, this being the distance chosen to represent wing length.

None of the comparisons of pairs of genotypes within environments is significant. It is noteworthy, however, that the difference between F_1 and P generations at 25° is the largest difference between two such pairs of observations and we have some grounds for feeling that a heterotic effect is present, although we cannot detect it. There are two reasons for this view. The first is that the test of significance utilized here is the analysis of variance and the error variance in all cases proved to be extremely large; hence our sample size of 100 individuals per genotype was probably too small and could well have been tripled. The second point is that the difference between the two wings of an individual fly is dependent to some extent upon the absolute length of the wings involved; the larger the wings, the greater the difference between wings irrespective of buffering ability.

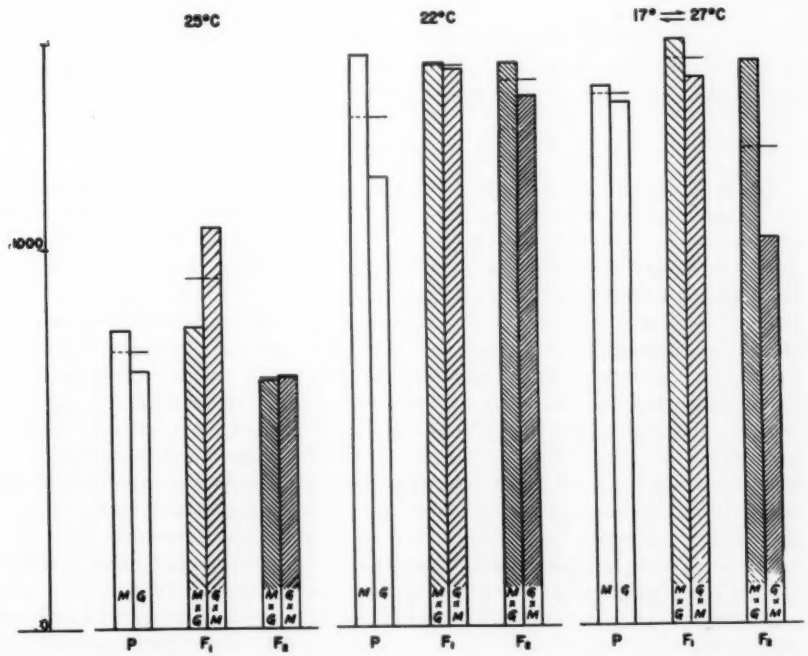

FIGURE 3.—Mean life egg production per female

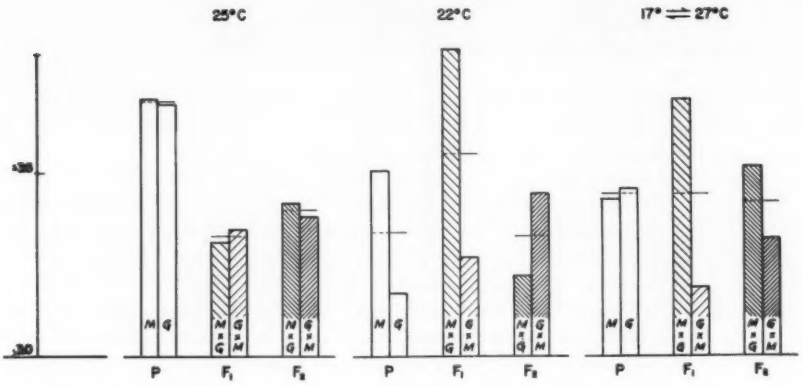

FIGURE 4.—Mean wing asymmetry per fly (in micrometer units).

Now the flies produced at 25°C were, of course, appreciably smaller than those produced in the other two conditions and this means that one unit of asymmetry at 25°C represents less efficient stabilization of development than does one unit of asymmetry at 22°C. It follows that the observed difference between F_1 and P at 25°C is indicative of a greater difference between homeostatic properties of the two generations than appears at first sight.

DISCUSSION

The data are summarized in Table 2. The + and − signs indicate the direction of difference in the comparisons P–F_1, F_1–F_2, P–F_2 and the figures are the probabilities for the various comparisons.

The results are interesting for several reasons. In the first place, they show that heterosis when it appears in F_1 hybrids is not necessarily found in all characters studied. The mean longevity of the flies is shown in Figure 5. It is apparent that there is no heterosis for this character in any environment. This is in contrast to findings of VETUKHIV (1957) whose work showed that F_1s between geographic populations exhibited the heterosis and F_2s showed the breakdown. This difference in experimental findings may not be very meaningful but it is possible that the difference is due to the fact that in the present study, the measurements of longevity were made on flies laying eggs more prolifically than in the original study, when the measurements were made upon flies kept under conditions not optimal for egg laying. The inference is that the energy in the one case is directed in the channel of egg production and in the other case into maintaining the antisenility defenses of the body and thereby promoting longevity.

We bring up this point for it does seem to us that here we may have alternative channeling of biological effort, the direction of which is dependent upon the immediate environment of the flies and not just a case of heterosis being shown in

TABLE 2

Significance level of differences between genotypes

	Viability	Eggs Per Day	Eggs Per Life	Fly Days	Wing Asymmetry	
$F_1 - P$	+ 0.01	+ < 0.05	+ < 0.05	+ > 0.20	− > 0.20	
$P - F_2$	− > 0.20	+ > 0.20	+ > 0.05	− > 0.20	+ > 0.20	25°
$F_1 - F_2$	+ > 0.20	+ < 0.05	+ < 0.01	− > 0.20	− > 0.20	
$F_1 - P$	+ > 0.20	+ > 0.05	+ > 0.20	− > 0.20	o > 0.20	
$P - F_2$	− > 0.20	+ > 0.20	− > 0.20	− > 0.20	+ > 0.20	22°
$F_1 - F_2$	+ > 0.20	+ > 0.05	+ > 0.20	− > 0.20	+ 0.20	
$F_1 - P$	− > 0.20	+ > 0.20	+ > 0.20	− > 0.05	− > 0.20	
$P - F_2$	− > 0.05	+ > 0.05	+ > 0.05	+ > 0.20	+ > 0.20	f° 17–27
F_1 F_2	− < 0.05	+ > 0.05	+ > 0.20	− > 0.20	+ > 0.20	

f° = F°.

same characters (viability and fecundity at 25°C) and not in others, e.g., longevity. Such situations have been noted by a number of workers, e.g., MORIWAKI *et al.* (1956), LEVINE (1952).

The second and more important point is that in this study it is quite clear that while in general the results obtained at 25°C agree with those utilizing geographic populations carried out previously, the same cannot be said of the 22° and F° (17/27) data. In the two latter environments we have no evidence for the occurrence of heterosis in crosses between the geographic populations used. For this reason we have presented no data on what DOBZHANSKY (1953) has called macroenvironmental variance. There is little doubt that if we did, we would not find the kind of result obtained by DOBZHANSKY and WALLACE (1953), i.e., F_1s (in their cases, chromosome heterozygotes) showing less macroenvironmental variance than homozygous parents.

We are led to consider that the lack of concordance between the different environments is simply a reflection of the relative stringencies of the environments used. A more stringent environment is closer to the biological limit for one or more factors, whether this be the thermal death point, oxygen tension, food supply, or any other. Now, as has been demonstrated by a number of workers and emphasized notably by LERNER (1954), euheterosis is connected with devel-

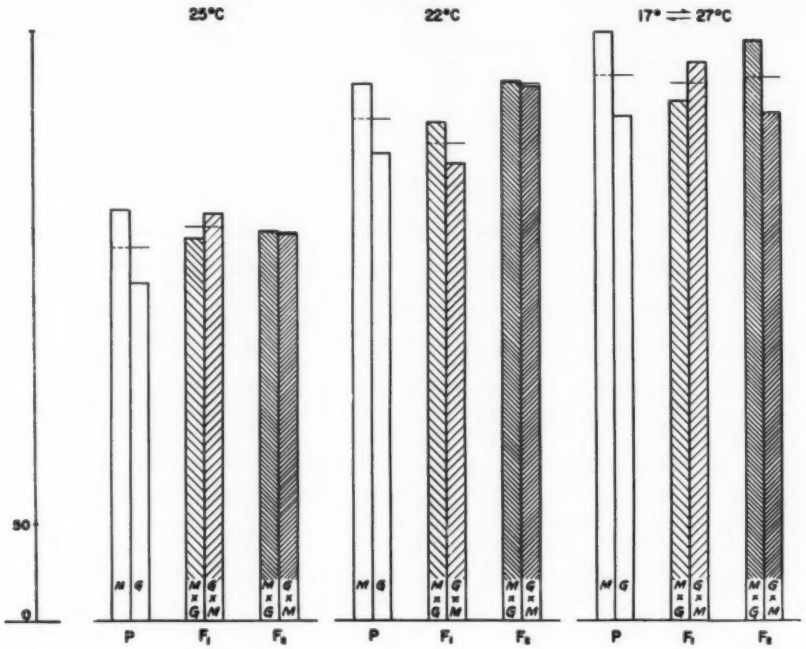

FIGURE 5.—Mean fly days.

opmental homeostasis, therefore, it seems logical to expect to find heterosis most *readily* manifested under stringent or otherwise unfavorable conditions. Of course, this does not imply that heterosis will not be seen under more nearly optimal conditions. There is some evidence to support this line of reasoning. Here we may mention the work of MORIWAKI *et al.* (1956) who investigated the comparative viability of gene-arrangement homozygotes and heterozygotes in *D. ananassae*. In two components of viability (prepupal and postpupal), both biologically significant indices of heterosis, no heterosis was observed under optimal conditions but was observed, "at least in part" under unfavorable or stringent condition.

We feel that it is legitimate to suppose that for *D. pseudoobscura* 25° C is a more stringent environment than is 22° C or the fluctuating condition 17° ⇄ 27° C. It produced the lowest mean viability of all genotypes taken together and the flies were less fecund. This means that heterosis is more likely to be manifested in this environment than in the others. It would be possible to alter some other components of the environment to produce stringency even in 22° C and „F"° conditions. If this were done we should then expect to find heterosis and possible

F_2 breakdown. The idea that the species in general is adapted to a temperature range in which 22° C is more optimal than 25° C has to be tested critically but is ostensibly sound. It is possible, however, that the populations used in these experiments possessed a special adaptation to low temperatures, for the strains of which they are composed have been maintained in the laboratory for many years at 15° C. A shift to 22° C is clearly less than a move to 25° C and this would mean that 25° C environment would in any case be more difficult or stringent than the other two.

The fundamental problem, however, why F_1 heterosis and F_2 breakdown are so often observed in experiments of the type described is still unsolved. The discussion by WALLACE and VETUKHIV (1955) postulated that interpopulational F_1 hybrids are superior to the parental populations by virtue not of heterozygosity alone, but also due to coadaptation of the entire genetic systems. To quote WALLACE and VETUKHIV (1955), "This heterosis must be ascribed to heterozygosity for different but integrated gene systems." Some components of coadaptation are involved here, however; notably those concerned, it appears, with the interaction of loci within a single chromosome, and perhaps, less importantly, those concerned with the relationship of different chromosomes in a haploid set. If a breakdown in F_2 is the usual result, then these components must be most affected, and the disruption of the coadapted system leads to a F_2 inferiority. On the other hand, what MATHER (1943) has called relational balance seems less important in our material.

SUMMARY

Some components of Darwinian fitness, i.e., larval viability, fecundity and asymmetry of the wings, have been studied in P, F_1 and F_2 of crosses between geographic populations of *D. pseudoobscura*. The three environments used were: 25° C, 22° C and a fluctuating temperature 17° to 27° C and back each 24 hours. The F_1 hybrids generally showed heterosis in their viability, fecundity, and in wing asymmetry at 25°, but not at 22° or under fluctuating temperatures. F_2 hybrid breakdown occurred in the same traits at 25°, but not in the other environments.

The results obtained suggest that heterosis may be manifested more readily under stringent than under optimal environmental conditions. Among the environments created in our experiments, the temperature of 25° C is presumed to be farther removed from the optimum for the flies than 22° or the fluctuating temperature cycle.

ACKNOWLEDGEMENT

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REVERSE MUTATION STUDIES ON THE FORKED LOCUS IN DROSOPHILA MELANOGASTER

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A SERIES of mutation experiments was undertaken in the hope of resolving some of the contradictory reports in the literature regarding the effectiveness of X-rays in producing reverse mutation in *Drosophila melanogaster*. Several workers, particularly PATTERSON and MULLER (1930), JOHNSTON and WINCHESTER (1934), TIMOFÉEFF-RESSOVSKY (1931, 1933a,b, 1939), and MULLER and OSTER (1957), have reported considerable success in inducing reverse mutation at various loci; whereas other studies (LEFEVRE 1950; KAUFMANN 1942; PITERNICK 1949; YU 1949; SPOFFORD 1955) on the same problem have been unproductive. Although these diametrically opposed results would, at first glance, seem to imply that one group of investigators has been seriously misled, perhaps because of inadequate techniques, the alternative conclusion that both kinds of results are correct cannot be ruled out. If there are two kinds of alleles, reversible and irreversible, then both positive and negative data may be expected in reverse mutation experiments. This possibility seems particularly plausible in view of the work of GILES (1951, 1953) and DE SERRES (1958) on reverse mutation in Neurospora.

Of interest is the fact that the recessive sex-linked mutant forked (*f*, 1–56.7) has consistently been reported in the positive Drosophila studies to be the most readily reversible of all mutants tested. Indeed, at one time TIMOFÉEFF-RESSOVSKY (1933a) claimed that X-ray-induced reverse mutation at the forked locus ($f \rightarrow f^+$) was equally as frequent as direction mutation ($f^+ \rightarrow f$). Later, to be sure, he apparently modified that conclusion, as may be inferred from a consideration of the mutation constants reported by TIMOFÉEFF-RESSOVSKY and DELBRÜCK (1936). Even in the later paper, however, reverse mutation of forked was calculated to be at least one third as frequent as direct mutation. More recently, MULLER and OSTER (1957) concluded that direct and reverse mutation rates at the forked locus, unlike those at other loci, are not very different from one another. Nonetheless, no X-ray-induction of reverse mutation at the forked locus was detected by KAUFMANN, LEFEVRE, or PITERNICK, even though sufficient numbers of gametes were tested to have produced several forked reversals if they had occurred at a rate comparable to that observed by TIMOFÉEFF-RESSOVSKY, JOHNSTON and WINCHESTER, or MULLER and OSTER.

In order to test the possibility that the divergent results at the forked locus

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stem from the existence of two different kinds of forked alleles (one reversible and used by investigators reporting the induction of reverse mutations; the other irreversible and used by investigators reporting negative results), a thorough study was undertaken of four different forked alleles whose capacity for spontaneous reverse mutation had been studied. The experiments carried out in this analysis yielded a most unexpected result. Following irradiation of mature sperm, none of the four *f* alleles tested on an extensive scale gave rise to induced f^+ reversals, whereas one particular allele, f^{3n}, produced a high frequency of apparently complete reversals when homozygous females, rather than males, were irradiated. Thus, instead of resolving the paradox of X-ray-induced reverse mutation in *Drosophila melanogaster*, the present study adds a new dimension to be considered in connection with the question of the mutagenicity of X-rays.

MATERIAL AND METHODS

The four *f* alleles chosen for study may be described as follows:

(1) f^1: The standard, original forked allele discovered by Bridges in 1912 (See Carnegie Publication **237**:58, and BRIDGES and BREHME 1944). f^1 responds to the forked suppressor of WHITTINGHILL (*su-f*, 1–64±). It reverts spontaneously at a rate of approximately 1/60,000 (See GREEN 1957).

(2) f^{36a}: A strong allele of spontaneous origin discovered by IVES in 1936 (See BRIDGES and BREHME 1944). It does not respond to WHITTINGHILL's suppressor of forked, and f^{36a} has not been observed to revert spontaneously. However, it recombines with f^{3n}, though not with f^1 (GREEN 1956).

(3) f^{51a}: An X-ray-induced allele produced by GREEN exhibiting strong expression like f^{36a} and is not suppressible; it has not been observed to revert spontaneously, nor has it been tested for recombination with other *f* alleles.

(4) f^{3n}: An allele similar in expression to f^1 that was extracted by GREEN from a triploid stock which, by its label and source, was presumed to have carried only f^1. Although it does not respond to WHITTINGHILL's suppressor (whereas f^1 does), other *f* suppressors will affect it. In large-scale experiments, it has been shown to revert spontaneously at the remarkably high rate of about 1/20,000 (GREEN 1957). Furthermore, it undergoes recombination with f^1 (GREEN 1955).

Four homozygous stocks were synthesized, each containing one of the four forked alleles, as well as vermilion (*v*) and carnation (*car*). In carrying out the male irradiations, *v f car* males less than three days old were placed in gelatin capsules and exposed to 5,000r, delivered by an X-ray machine operating at 250 KV and 15 ma. At a target distance of 35 cm, unfiltered, the dose rate was about 420r per minute. After exposure, the males were mated to virgin females that were homozygous for the sex-linked gene markers y^+, w^a, *v*, and *f*, as well as for two inversions, scute-8 and delta-49. This stock, originally received from H. J. MULLER, will hereafter be referred to as the M-6 stock. All F_1 daughters were

carefully examined for the presence of straight, wild-type bristles, as well as for any changes in eye color or body color. The mating period was limited, in most experiments, to six days, thus restricting the test to the ability of X-rays to produce *f* reversions in mature sperm.

When females rather than males were irradiated, three-day-old virgin *f* females were exposed, as above, to a dose of about 4,000r. The irradiated females were then mated to M-6 males, and thereafter the parent flies were subcultured at intervals in order to test the reversibility of the *f* allele in female germ cells at successively more immature stages.

In some experiments, attached-X, rather than free-X, females were irradiated with an X-ray machine operated at 100 KV, 30 ma, filtered with ½ mm Al. The dose rate varied from about 700 to 900r per minute.

In the case of f^{3n} in particular, homozygous *v* f^{3n} *car* females were exposed to 4,200r and mated individually. Each surviving fertile female was subcultured ten times over the course of four weeks. At the same time, f^{3n} males were exposed to the same X-ray beam and subcultured on a similar schedule, thus providing a comparable test of the relative mutability of f^{3n} in male and female germ cells.

RESULTS

A. Male exposures: The results of the male exposures are summarized in Table 1. In all cases, the irradiated males were mated in mass cultures, and on the whole the experimental bottles were rather heavily populated. For comparison with the results of the irradiation experiments, control data on the spontaneous mutability of f^{1} and f^{3n} as reported by GREEN (1957) are recorded. These data represent the spontaneous reverse mutability in females, rather than in males, and thus do not provide fully comparable controls. However, in truly male control experiments, no reversals appeared in tests of 14,020 gametes.

From inspection of Table 1, it is clear that the incidence of reverse mutation in the irradiated cultures is no higher than in the controls. It should be emphasized that only completely wild-type reversions, in contrast to partial reversions, are recorded in the table. Although these data do not support the view that X-rays are effective in producing reverse mutations, they emphasize the fact that certain

TABLE 1

Reverse mutation at the forked locus following irradiation of mature sperm with 5,000r

Allele irradiated	No. loci tested	No. f^{+} reversions	Reversion rate
f^{1}	66,000	1	1.5×10^{-5}
f^{3n}	74,000	2	2.7×10^{-5}
f^{36a}	46,600	0	----------
f^{51a}	45,500	0	----------
		Controls	
f^{1}*	457,300	7	1.5×10^{-5}
f^{3n}*	298,700	14	4.7×10^{-5}
f^{36a}	91,600	0	----------

* Control data from GREEN (1957).

f alleles exhibit a sufficiently high spontaneous reverse mutability to account for the sporadic occurrence of f^+ mutants among the offspring of irradiated forked males. Furthermore, f^{3n} in all likelihood arose in a culture that was originally f^1, or at least was never identified by its stock designation as being other than f^1 (see discussion by GREEN 1957) until its pseudoallelic difference from f^1 was discovered. Thus, reports of a high spontaneous reverse mutability of *f* by RHOADES (1931) and LINDSLEY (1954) indicate that, in reality, f^{3n} (or a similar allele) may be widespread in stocks thought to be f^1.

B. Female exposures: When forked females, rather than males, are exposed, a quite different picture emerges. Data of GREEN (1959) reveal that f^+ reversions appear at a remarkably high rate following irradiation of f^{3n} in females; however, no more reversions than would be expected spontaneously occur following irradiation of f^1 in females. GREEN's data are recorded in Table 2. It might be noted

TABLE 2

Reverse mutation following X-radiation of f^1 *and* f^{3n} *attached-X females with 4,000r (Data from GREEN 1959)*

Allele irradiated	No. loci tested	No. f^+ reversions	Reversion rate	Control rate
f^1	68,268	1	1.5×10^{-5}	1.5×10^{-5}
f^{3n}	115,774	38	32.8×10^{-5}	4.7×10^{-5}

that these results were obtained in mass cultures, and few if any of the reversals were identified as clusters despite the fact that many of them must have originated in oogonial cells rather than in oocytes.

Since it appears that the capacity of f^{3n} to revert following irradiation of males differs so markedly from its capacity to revert following irradiation of females, a special experiment was devised in order to determine if the difference is real or is produced as a result of different procedures, techniques, or biases of different investigators. Three-day-old homozygous *v* f^{3n} *car* females were irradiated with 4,200r, mated individually to M-6 males, and subcultured every two or three days for their entire fertile lives. Simultaneously, *v* f^{3n} *car* males were exposed in the same X-ray beam, mated to M-6 females, and also subcultured repeatedly. The results of this experiment are recorded in Table 3.

Several facts emerge from a study of Table 3. First, markedly increased numbers of complete forked reversals appeared beginning immediately after irradiation of f^{3n} females. During the first week, mutations occurring in oocytes gave rise to the reversals listed. During the second week, and thereafter, oogonial mutations were the source of the reversals. Clusters of f^+ individuals started appearing in the second week, and altogether the 37 reversals shown as occurring in clusters were produced by eight separate irradiated females. The average cluster size, then, was 4.6; the largest cluster contained eight individual mutants. It may be noted that, in the female series, the incidence of f^+ reversals remained relatively constant from the earliest to the last subculture.

TABLE 3

Incidence of complete f+ *reversals produced in successive subcultures following simultaneous exposures of* v f^{3n} car *males and females to 4,200r*

	No. loci tested	No. f^+ reversions found single	as clusters	all	Reversion rate
		f^{3n}/f^{3n} females irradiated†			
1st week	10,830	5	0	5	46.2×10^{-5}
2nd week	37,257	5	18	23	61.7
3rd week	23,650	1	14	15	63.4
4th week	10,023	0	5	5	49.9
Total	81,760	11	37	48	58.7×10^{-5}
		f^{3n} males irradiated			
1st week	14,309	0	0	0	----------
2nd week	1,889	0	0	0	----------
3rd week	8,369	0	7	7	83.6×10^{-5}
4th week	5,984	0	1	1	16.7
Total	30,551	0	8	8	26.1×10^{-5}
					(55.7×10^{-5})*

* Reversion rate in spermatogonial stages only.
† Irradiated females were mated individually in all cases, but the majority of irradiated males were subcultured in mass. (Data summarized by weeks, although two or three subcultures were actually made each week.)

In the case of the irradiated males (which were exposed simultaneously with the females and were subcultured on a similar schedule), a rather different picture emerged. No reversals were noted in the first two weeks, confirming the real difference in mutability of f^{3n} in postmeiotic sperm stages as compared with its mutability in female germ cells. However, after the second week, one cluster of eight mutant individuals appeared, unquestionably as the result of a spermatogonial mutation.

Both spontaneous and induced reverse mutations of vermilion and carnation were detectable in most of the experiments recorded above. However, not one case of reversion at either locus was detected.

DISCUSSION

The results of the present experiment clearly point to the lack of equivalence in the mutational results, under certain circumstances, of irradiating males and females. The reality of a significant difference in mutational response of f^{3n} in mature sperm, as compared with its response in oocytes and oogonia, has been fully demonstrated, but the cause for the different response is far from clear. However, an obvious difference that may well be related to the divergent results is that mature sperm are haploid (although spermatocytes and spermatogonia are diploid), whereas even the most mature female germ cell that can be irradiated is diploid. Moreover, many biochemical differences between sperm and egg surely exist, any of which may influence mutability.

The situation here is reminiscent of that encountered in crossing over. No crossing over occurs during spermatogenesis in Drosophila, whereas crossing over

occurs freely during oogenesis. Is the reversibility of f^{3n} in the female somehow correlated with crossing over, an event which does not transpire in the male? Anything analogous to pseudoallelic recombination must be ruled out as an explanation for the production of wild-type mutants in the experiments where homozygous (f^{3n}/f^{3n}) females were irradiated. Likewise, gene conversion in the ordinary sense cannot account for the results. Whether or not atypical recombinational events can produce f^+ from f^{3n}/f^{3n}, but not from a single f^{3n} allele, is a matter for speculation. Surely, the rate of production of f^+ following female irradiation is sufficiently high (more than one in 2,000) to suggest a recombinational, rather than a mutational, explanation.

If recombination were somehow related to the formation of the f^+ mutants, it might be predicted that radiation of spermatogonial stages would be effective, even if irradiation of mature sperm were not. Those experiments in which f^{3n} males were irradiated simultaneously with females and subcultured for four weeks permitted a test of mutability in the spermatogonial stages. Indeed, one cluster of f^+ reversals was observed beginning in the third week. However, this case may well have resulted from a spontaneous mutation in a diploid spermatogonium rather than from an X-ray-induced reversion. No other information is available.

All of the reversals reported here appeared to be completely wild type, having fully normal bristles, and those tested thoroughly exhibited completely normal crossover relationships with neighboring loci. Relatively few mutants that might have been called partial reversals were observed, and none have been included in the tabulated results. Furthermore, no fractional reversals were detected. For the most part, the apparent partial reversals that were saved for breeding tests did not breed true, and after one or two generations were indistinguishable from unmodified f^{3n}. Some suppressors of f^{3n} were detected, however.

Since the cellular environment of the f^{3n} gene seems to affect the ability of X-rays to induce reversions, it may be that still other types of cellular environments would also modify the mutational response of forked alleles to irradiation. Thus, MULLER and OSTER (1957) irradiated mature sperm in the spermathecae of females, and reported increased rates of reversions compared to unirradiated controls. Actually, however, their data on f^1 differs from ours primarily in the much lower control rates that they detected and in their inclusion of partial as well as complete reversions. Eliminating the partial reversions from their data would leave their induced reversion rates for f^1 (and f^2, which we did not test) little higher than our control rates for f^1 (3 per 75,000 as compared with 7/457,000) and not as high as our control rate for f^{3n} (14/298,000). Although the spermathecal environment may have a real effect on mutability, it certainly does not produce as pronounced an effect as that found in the female germ cell itself.

The existence of irreversible alleles seems to be well established. In our experiments neither f^{36a} nor f^{51a} reverted spontaneously or after irradiation. The same is true of f^r, studied by MULLER and OSTER. On the other hand, certain f alleles, such as f^{3n}, exhibit such unusually high spontaneous reverse mutation rates that,

in the absence of adequate controls, the occurrence of wild types in a radiation experiment would be sufficiently numerous to warrant the conclusion that they were induced by the irradiation. However, the remarkable allele specificity seen at the forked locus makes prediction of mutation rates impossible. Although f^{3n} does not respond to irradiation in mature sperm, it does so in oogonia, oocytes, and probably also in spermatogonia. In contrast, f^{1}, although spontaneously reversible, does not exhibit increased mutability in any sperm or egg stage after irradiation. These various facts make it unlikely that the earlier results of X-ray-induced mutation in Drosophila fell into two categories because of the chance use of irreversible alleles by one group of investigators and reversible alleles by the other. Whatever the true cause may have been, another type of explanation must be sought.

In virtually all of the experiments reported here, reverse mutations of vermilion were detectable and, in many cases, of carnation as well. However, not one spontaneous or induced reversion of either *v* or *car* was found, regardless of whether males or females were tested. This fact clearly emphasizes the unusual behavior of the forked locus, and makes even more uncertain predictions about the mutability of untested alleles.

SUMMARY

X-rays are generally ineffective in increasing reverse mutation rates at the forked locus. However, in the specific case of f^{3n}, an unusually high reversion rate is observed both spontaneously and following irradiation of oogonia and oocytes, but not after irradiation of postmeiotic stages in the male. Evidence regarding spermatogonial stages is inconclusive at present.

The reversion rate of f^{3n} to f^{+} observed in the female irradiations varied from about 1/1700 to 1/3000. Rates of this magnitude seem more suggestive of recombinational phenomena than of reverse mutation.

The existence of both reversible and irreversible alleles at the forked locus seems well established. This fact, coupled with the remarkable mutational specificity exhibited by different forked alleles, makes it completely impossible to make intelligent predictions about mutation rates of untested alleles. However, the divergent results of earlier workers on X-ray-induced reverse mutation in Drosophila do not seem to be understandable simply by assuming that some investigators happened to use reversible alleles, others irreversible.

The failure to observe any reversions at the vermilion and carnation loci emphasizes the more usual behavior of typical loci in reverse mutation studies in Drosophila.

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THE RELATIONSHIP OF HETEROZYGOSITY TO HOMEOSTASIS IN MAIZE HYBRIDS[1]

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BOTH likeness and constancy are implied by the word homeostasis. In the genetic context, genotypes which interact with a variable environment in such a way as to maintain relative uniformity of an observable characteristic therein fulfill both the spirit and the letter of the concept, homeostasis. Though primarily morphological characteristics have been studied, their stability of development in a variable environment implies physiological adjustments. The capacity for such adjustments is a property of the homeostatic genotype.

DEMPSTER (1956), commenting on LEWONTIN's (1956) use of the term homeostasis, and citing CANNON's original definition, states "It seems clear . . . that homeostasis applies to particular conditions that are maintained in a relatively stable state by special mechanisms that have evolved through natural selection; whereas the causative conditions, which are themselves variable, are called homeostatic devices or mechanisms. Homeostatic conditions seem important aids to, or components of, fitness, but not fitness itself."

GRAFIUS (1956) has studied a theoretical model involving this concept and presented experimental data in its support. Grain yield in oats (*Avena sativa* L.) was represented geometrically as a rectangular parallelepiped of volume W and with edges X (number of panicles per unit area), Y (average number of kernels per panicle), and Z (average kernel weight); thus $X \cdot Y \cdot Z = W$. A universal variety (the homeostatic genotype) was defined as a variety which equals or exceeds the mean yield at all locations in a region. On the basis of the theory developed, among other properties of the universal variety, *changes in X, Y, and Z would tend to counterbalance;* and the edge most subject to change would be the longest, the edge least subject to change, the shortest. GRAFIUS concluded from this analysis that grain yield of some genotypes may remain relatively invariant over a range of environments even as the components of yield vary. The changes in the components are compensating such that their product ($X \cdot Y \cdot Z$) is relatively invariable. The components of yield are homeostatic devices, in the sense of CANNON (DEMPSTER 1956), which assure homeostasis of yield. Homeostasis can be measured in terms of relative variability of a characteristic over a range of environments.

In maize, single cross hybrids are generally more homeostatic for yield and other adaptive traits than are the parental inbred lines (SHANK and ADAMS 1959).

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Two explanations have been advanced to account for superior homeostasis or buffering in heterozygous individuals (THODAY 1955). The first attributes a special property to heterozygosity that promotes versatility in development beyond that possible in homozygotes. The second view attributes no special property to heterozygosity except the classic one of promoting segregation, which leads to superior buffering in hybrids by virtue of the heterozygous balance achieved in outbreeding species by natural selection.

Results bearing on this problem are presented from an experiment on environmental variability of maize hybrids which were produced so as to possess different levels of heterozygosity.

MATERIALS AND METHODS

Random plants of two locally grown open pollinated strains of maize, ELLIS and ARMSTRONG, were initially self-pollinated in 1949, and individual plants selfed each year thereafter through five successive generations to establish a group of nearly homozygous inbred lines. Related lines were produced during the inbreeding phase by selfing sibling plants. Depending upon the stage of inbreeding when sublines were started, some lines were closely related, others were related to a lesser extent, and some were related only through having descended from a common noninbred plant in the open pollinated source strain. From this material it was possible, in 1955, to recombine in single crosses inbred lines possessing a high level of homozygosity within lines, but of varying degrees of relationship between lines. On this account the proportion of heterozygous loci to be expected in the hybrids could be expressed as a function of the calculated inbreeding coefficients of the hybrids themselves.

The hybrids were arrayed in a total of eight groups, differing in level of heterozygosity. Hybrids in groups 1 through 5 were produced with expected inbreeding coefficients (F) of 0.969, 0.923, 0.861, 0.738, and 0.492, respectively. It was assumed that heterozygosity in the hybrids would be proportional to F from the relation $H = 1 - F$. Implicit in the assumption are that (1) no effects result from differential genetic homozygosity of the basic populations, and (2) no natural or artificial selection favoring heterozygosis or particular genotypes occurs during the inbreeding process. In terms of level of heterozygosity, calculated as $H = 1 - F$, the first five groups of hybrids had expected values of 0.031, 0.077, 0.139, 0.262, and 0.508, respectively. Group 6 was composed of intravarietal hybrids from inbred lines related only in having been derived from different open pollinated plants of a particular source variety. Group 7 was made up of intervarietal hybrids, the two inbreds in each case being derived from different source varieties. The hybrids in group 8 were formed from Corn Belt inbreds of diverse origins.

Since the expected level of heterozygosity (H) is based on the inbreeding coefficient, and is therefore relative to the open pollinated source variety as a base, the intravarietal crosses of group 6 were considered to have $F = 0$, or $H = 1$. However, it is reasonably to be expected that the intervarietal crosses of group 7

should be more heterozygous than those of group 6; whereas the level of heterozygosity of crosses in group 8 is probably the highest of all groups. The assignment of relative values of expected level of heterozygosity to groups 7 and 8 is of necessity somewhat arbitrary, though there is sufficient reason for believing them to be arrayed in the correct order of magnitude.

It is recognized that after five generations of selfing complete homozygosity will not have been attained in a diploid organism. The approximately three percent residual heterozygosity in the inbred parents presumably would manifest itself as genetic variance in the hybrid. But this effect would be common to hybrids in groups 1 through 7, and thus could not influence comparisons involving these groups, unless it happened that one or more genes segregating in an otherwise nonsegregating background generated variance as some function of the level of heterozygosity of the rest of the genotype. Group 8 would be less subject to the foregoing qualification. With the acknowledgment of possible trivial bias from this source, it should be clear that in each F_1 all full sib plants were essentially genetically identical, and differences among them could be attributed almost wholly to environmental causes.

The material was grown in 1956 at Brookings on Vienna loam, a relatively uniform upland glacial till soil, and at Menno on a soil complex of variable zonal and solonetz phases derived from glacial till. In 1957 the test was grown again at Brookings, and at Highmore on a variable loam-clay complex derived from glacial till.

In 1956 there were 34 hybrids, and in 1957, 40 hybrids in the field trials. In all locations the plots were single rows 30 hills in length with plants spaced 14 inches apart in the row. Each hill contained a single plant, and each test row was grown between two rows of an adapted single cross hybrid to provide uniform competition throughout the test. Measurements were not taken on plants located at the ends of the row or on plants adjacent to missing hills; otherwise the measured plants were taken successively in the row. Ten plants per plot were measured in the field, the traits being plant height, flag leaf height, and ear node height. At maturity, but before loss of leaves, the ten plants previously measured were cut close to the ground, chopped into short lengths, individually bagged, dried, and weighed. The ear from each plant was then husked and weighed separately.

Coefficients of variation were determined on each plot for each trait. Means of plots were compared by analysis of variance, and the relationship of group means over years and locations to expected level of heterozygosity was evaluated by regression. The levels of heterozygosity calculated for groups 1 through 6 followed a geometric progression so the values of H for all groups were transformed to logarithms for purposes of determining the regression of buffering capacity on heterozygosity.

EXPERIMENTAL RESULTS

Average values of the coefficients of variability for each level-of-heterozygosity group for each character in each environment are presented in Table 1. In each

TABLE 1

*Mean values of coefficient of variability for each level-of-heterozygosity group for each trait in each year and locality**

Trait	Heterozygosity group number 1	2	3	4	5	6	7	8
Plant height								
Brookings 1956	7.62	6.65	7.12	5.67	5.01	5.03	4.81	4.69
Brookings 1957	7.47	6.73	6.95	5.90	6.66	4.90	6.71	6.67
Menno 1956	10.44	9.73	8.68	9.54	9.43	6.68	6.98	7.72
Highmore 1957	11.21	9.81	9.20	8.89	8.89	5.75	6.12	6.95
Flag leaf height								
Brookings 1956	8.82	7.99	8.64	6.97	5.68	6.13	5.90	5.52
Brookings 1957	8.67	7.70	8.23	7.10	7.70	5.99	7.80	8.55
Menno 1956	12.28	11.83	10.43	12.22	11.43	8.40	8.36	8.75
Highmore 1957	13.27	11.47	10.59	9.94	9.97	6.66	7.01	7.11
Ear node height								
Brookings 1956	14.53	12.91	16.32	14.16	9.85	9.65	11.03	6.03
Brookings 1957	18.67	11.97	13.68	11.71	10.01	10.20	9.84	11.42
Menno 1956	16.85	15.52	18.74	17.09	13.63	12.10	13.74	10.93
Highmore 1957	20.14	18.50	17.72	17.49	18.15	12.41	11.71	11.20
Total plant weight								
Brookings 1956	19.94	21.48	19.65	19.33	16.20	17.07	16.89	12.05
Brookings 1957	25.22	23.53	18.98	21.93	19.57	20.43	21.67	17.01
Menno 1956	30.01	25.81	23.50	30.21	21.27	23.40	24.46	18.94
Highmore 1957	29.77	27.52	25.01	25.22	20.51	22.69	21.28	14.67
Ear weight								
Brookings 1956	32.87	30.56	25.05	26.62	18.44	15.42	18.10	14.40
Brookings 1957	41.77	38.81	33.65	40.59	25.06	22.49	26.68	19.41
Menno 1956†								
Highmore 1957	36.97	38.59	33.68	37.35	18.18	17.58	20.87	14.96

* 1956: Group 1 included six hybrids; groups 2 through 8 included four hybrids per group.
1957: Groups 1 through 8 included five hybrids per group.
† Data not usable on account of the predominance of barren plants throughout the experiment.

environment and for all traits but one, the heterozygosity groups differed significantly in buffering capacity, the single exception being flag leaf height at Brookings in 1957. In most instances hybrids within levels of heterozygosity also varied significantly in buffering ability (Table 2).

In Table 3 are given the mean coefficients of variation, averaged over all levels of heterozygosity, for each trait, year, and location. These indicate that variability was generally least at Brookings, which was characterized by a more uniform soil and favorable climatic environment, with one notable exception, ear weight in 1957.

The mean coefficient of variation of each group, for each trait in turn, was plotted against the logarithm of the calculated level of heterozygosity for that group (Figure 1). The relationship in each case obviously is essentially linear, and this is conclusively demonstrated in Table 4. From 75 to nearly 90 percent of the variation in buffering capacity can be attributed to the level of heterozygosity of the groups.

TABLE 2

Significance of differences in buffering capacity between and within groups of maize hybrids

Trait	Brookings 1956	Brookings 1957	Menno 1956	Highmore 1957
Plant height				
Between groups	***	*	***	***
Within groups	***	***	N.S.	N.S.
Flag leaf height				
Between groups	***	N.S.	***	***
Within groups	***	*	N.S.	N.S.
Ear node height				
Between groups	***	***	***	***
Within groups	***	***	***	N.S.
Total plant weight				
Between groups	***	***	***	***
Within groups	***	***	**	*
Ear weight				
Between groups	***	***	------†	***
Within groups	N.S.	*	------†	***

* P≦0.05.
** P≦0.01.
*** P≦0.005.
† No data obtained.

TABLE 3

Mean coefficients of variation for locations and years, averaged over all levels of heterozygosity, for each trait

Trait	1956 Brookings	1956 Menno	1957 Brookings	1957 Highmore
Plant height	5.94	8.75	6.50	8.35
Flag leaf height	7.07	10.57	7.72	9.50
Ear node height	11.97	14.94	12.19	15.92
Total plant weight	17.95	25.01	21.04	23.33
Ear weight	22.68	------†	31.06	27.27

† No data obtained.

TABLE 4

Linear regression of coefficient of variability on log heterozygosity for each trait

Trait	Linear regression coefficient	Percent of variability due to regression on heterozygosity
Plant height	— 1.48**	80.267
Flag leaf height	— 1.79**	80.538
Ear node height	— 3.58**	88.296
Total plant weight	— 4.02**	75.492
Ear weight	—10.62**	81.606

** P≦0.01.

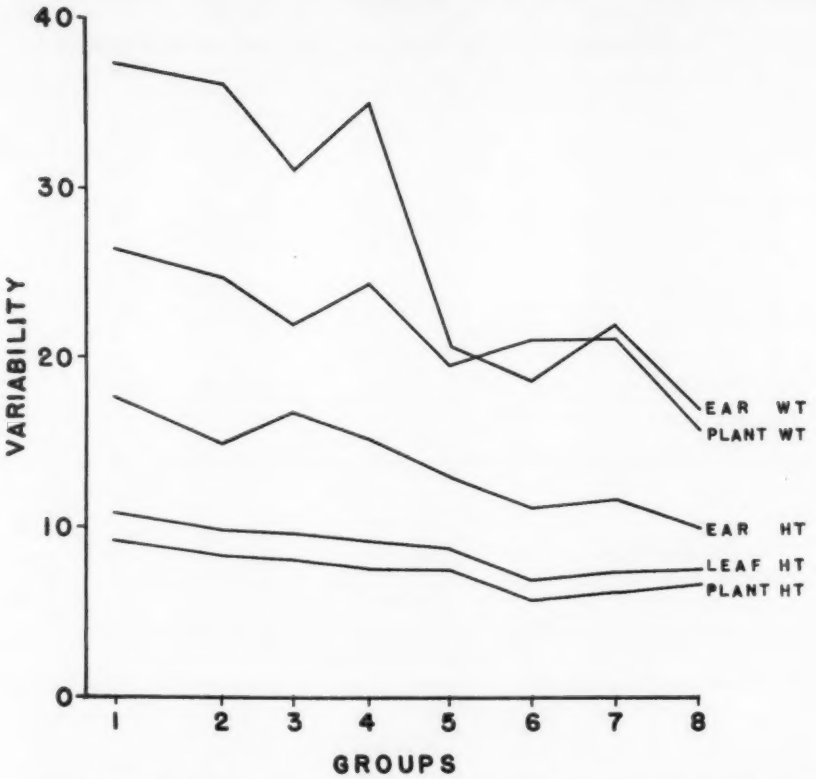

FIGURE 1.—The relationship of coefficients of variability to log heterozygosity in eight groups of hybrid maize. Groups, as plotted on the abscissa (3-cycle log scale), are given in order of increasing heterozygosity. Variability, plotted on the ordinate, is inversely proportional to homeostasis.

Taken collectively, these data show highly significant linear relationships existing between the level of buffering and the degree of genetic heterozygosity, with high levels of buffering being associated with high levels of heterozygosity. The traits related to length, such as plant-, flag leaf-, and ear node-height, that developed during the earlier more favorable part of the growing season show small regression values and less fluctuation from one level of heterozygosity to another.

DISCUSSION

There was obtained in this experiment compelling evidence of a major dependence of buffering capacity on level of heterozygosity of the groups of maize

hybrids studied. The evidence does not specify whether it is genes for height and weight that must be heterozygous to confer homeostasis, or whether the heterozygosity could refer to "background" genes not involved directly in the immediate events leading to height and weight traits.

In either case the evidence is in accord with the hypothesis that the increased biochemical versatility assumed to be possessed by plants by virtue of the heterozygous state of certain of their genes confers on such genotypes superior homeostasis.

But it has been observed in the present experiment and previously (SHANK and ADAMS 1959) that hybrids belonging to the same level-of-heterozygosity group frequently manifest significantly different buffering properties. These results clearly indicate that heterozygosity *per se* is not the only hypothesis required to account for homeostasis.

LEWIS (1954) supported the idea that biochemical versatility would be manifest, at least in part, in alternative pathways of biosynthesis functional under different environmental optima. Similarly, HALDANE (1954) advanced the possibility that a complex enzyme (presumably gene controlled) whose constituent parts possessed sharp optima for environmental variables would be of definite advantage in an organism subjected to a wide range of environments. Such an enzyme might result from the action of one or more genes in the heterozygous state, or from the simultaneous presence of two or more interacting alleles at different loci; and it is thus a potential property of both heterozygotes and homozygotes. But because they possess a greater number of different alleles, and hence a greatly enhanced frequency of potential allelic and nonallelic interactions, heterozygotes in maize are likely to be more highly buffered than are homozygotes. Differential buffering in homozygotes would depend upon their possessing, in greater and lesser degrees, genetically fixed nonallelic interactions (epistasis).

In a gene pool of a normally outbreeding species the organization of genes may be relationally balanced or coadapted (MATHER 1953; DOBZHANSKY and WALLACE 1953). If lines are extracted from this kind of population, made homozygous, and hybridized so as to produce single crosses of predetermined levels of heterozygosity, it might be expected that buffering capacity of the hybrids would increase proportionately with increasing heterozygosity to the level of the random mating population in approximate equilibrium, but thereafter decrease with further increases in heterozygosity.

A priori it might be assumed that the random mating population in approximate equilibrium is represented most closely in maize by the locally adapted open pollinated variety. If that were true, the intravarietal F_1's of group 6 on the average should be expected to restore the level of heterozygous balance which had been achieved by natural selection in that population before inbreeding was commenced. The intervarietal hybrids of groups 7 and 8, however, should be less well buffered because the fine adjustments leading to heterozygous balance in a common gene pool had not been achieved, owing to the recent reproductive isolation of the parental source populations.

Any interpretation to be made at this point must rest on the validity of the *a priori* assumption stated above. Since the work of BEAL at Michigan, corn breeders have known that crosses between open pollinated varieties might be heterotic for yield. In a recent experiment involving six North Carolina open pollinated varieties crossed in all possible combinations the average increase of crosses over the midparent amounted to 19.9 percent (ROBINSON *et al.* 1956). The genetic differences between varieties were attributable either to inbreeding due to finite population sizes or to divergent mutational changes in noninterbreeding populations. The authors considered it entirely plausible that the varieties comprised partially inbred populations. This was in agreement with the viewpoint of SPRAGUE (1955), referring to the status of open pollinated varieties in general. They also questioned whether the open pollinated variety is in equilibrium at all.

The evidence, though not conclusive, fails to uphold the assumption that the partially isolated, locally grown, open pollinated variety of maize is the random mating population in approximate equilibrium demanded by the heterozygous balance hypothesis. Rather, it is to be assumed that the recent reproductive isolation has been insufficient either in time or space, to bring about new heterozygous balance, and that variety crosses tend to restore the balance previously achieved by natural selection in the larger population.

The present experiment, then, is concerned in groups 7 and 8 with crosses representing that larger population, that is, with crosses derived from inbred lines out of numerous partially isolated and partially inbred open pollinated varieties, which more nearly tend to restore the state of natural heterozygous balance. On this view, the regression analysis, in accounting for 75 to 88 percent of the variation in buffering by dependency upon heterozygosity, is in satisfactory agreement with the heterozygous balance concept.

By analogy with the geometric representation of a complex trait (GRAFIUS 1956), these results may also be interpreted to suggest that there exist back of each trait studied component processes or characteristics which, if experimentally observed, would be found to vary with fluctuating environment in such a way as to maintain relative constancy of the initial complex trait. If this were true it would be meaningless to discuss the heterozygosity of genes for the complex trait itself, for such genes may not in fact exist. The genes whose biochemical versatility has been inferred may function primarily to adjust the component processes in a compensating fashion. These processes correspond to the special mechanisms or homeostatic devices of DEMPSTER (1956) which are themselves variable but which thereby insure relative stability of particular conditions like height or weight.

It can be suggested further that these processes shift in a compensating fashion because of natural selection for stability of the complex trait, and that heterozygosity is one means of conferring the needed flexibility upon the component processes.

The dependency of homeostasis on heterozygosity in maize hybrids is paralleled

by the relationship of heterosis to heterozygosity. The components hypothesis discussed in this paper as an alternative explanation of homeostasis has previously been proposed as the basis of a new definition of heterosis (GRAFIUS 1956). It was recently shown (GRAFIUS 1959) that 50 percent of the heterosis exhibited in barley hybrids could be accounted for by interactions of three components of grain yield. On this hypothesis, both phenomena, homeostasis and heterosis, result from one fundamental geometry of enumeration and measurement. The genetic system controlling variation of the component processes, however, need involve only the classic concepts of additivity, dominance, and epistasis.

SUMMARY

Homeostasis was measured in eight groups of maize hybrids which had been produced so as to achieve greatly different levels of genetic heterozygosity between groups. Thirty-four hybrids in 1956 and 40 hybrids in 1957 were evaluated at two locations during each year for homeostasis in five traits by comparison of intraplot coefficients of variation.

Evidence of two kinds was obtained. First, homeostasis in these maize hybrids was highly related to the expected levels of heterozygosity of the hybrid groups. For the five traits studied, namely, plant height, flag leaf height, ear node height, total plant weight and ear weight respectively, 80.3, 86.5, 88.3, 75.5, and 81.6 percent of the differences in buffering capacity were attributable to differences in heterozygosity of the eight groups of hybrids. Second, differences between hybrids within groups were, in most cases, highly significant, which suggests that intrinsic heterozygosity is not a sufficient explanation of homeostasis in maize.

Any proposals aimed at explaining homeostasis must account both for the paramount role of heterozygosity, and for the differences in buffering manifested by inbreds, and by hybrids at the same level of heterozygosity.

Three hypotheses are discussed; they are not necessarily mutually exclusive. The first one postulates either the existence of alternative pathways of biosynthesis, optimally functional under different environmental conditions, and depending on heterozygous loci or nonallelic gene interactions, or the presence of a complex enzyme whose constituent parts function alternatively in a fluctuating environment. In theory this proposal seems feasible, but in practice it presents formidable difficulties in the designing of experiments affording a critical test. The data obtained in the current study are consistent with it but are not critical so far as this hypothesis is concerned.

The heterozygous balance hypothesis, with certain assumptions, leads to the prediction that homeostasis should increase with increasing heterozygosity, at least up to the level of heterozygosity established in the random mating equilibrium population. To a satisfactory degree the prediction was fulfilled by the data of this study. The hypothesis, however, fails to account readily for evidence of the second kind, namely, differences in buffering among inbreds or among hybrids at the same level of heterozygosity.

In the third hypothesis it is proposed that back of each major homeostatic trait

are to be found component characteristics or processes which vary in a compensating pattern to promote relative constancy of the complex trait. The flexibility of the components is derived from the biochemical versatility conferred by heterozygosity. But nonallelic gene interactions are capable of conferring differential versatility on homozygotes, and so both kinds of evidence can be answered in this hypothesis. A direct test of this hypothesis was not sought in the present study, but it may be readily tested.

Comparing the first and third hypotheses, it appears that in either case heterozygosity promotes internal adjustment to gain external stability. It is not clear, however, whether adjustments are made at the genic level or at the level of component variation.

The relationship of homeostasis to heterozygosity is analogous to the relationship of heterosis to heterozygosity and, on the components hypothesis, both phenomena might result from a common fundamental geometry of enumeration and measurement.

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INFLUENCE OF GENES CONTROLLING FLOWER COLOR ON RELATIVE QUANTITIES OF ANTHOCYANINS AND FLAVONOLS IN PETALS OF IMPATIENS BALSAMINA[1]

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IN earlier studies concerned with the genetic regulation of pigmentation in the garden balsam, *Impatiens balsamina*, the genes which determine the nature of anthocyanin pigmentation were found in individual cases to regulate also the structure of leucoanthocyanins (ALSTON and HAGEN 1958) and flavonols (CLEVENGER 1958). Thus the dominant gene *H*, while increasing the quantity of pelargonidin derivatives present in the flowers, also allows production of a leucopelargonidin, which does not occur when the recessive allele, *h*, is homozygous. At another locus, *L* governs production of anthocyanins derived from malvidin and at the same time introduces a polyhydroxy flavonol, apparently myricetin. The simplest interpretation of these observations would indicate that an intermediate made available for anthocyanin synthesis may also be exploited for leucoanthocyanin and flavonol synthesis and, therefore, that both *L* and *H* operate at an early stage in the elaboration of the flavonoid structure.

Additional control of flower color in the balsams is provided by a series of alleles *p*, P^g and P^r. While slight anthocyanin pigmentation may occur with *p*, the principal influence of these genes seems to be an augmentation of anthocyanin production, *p* being the least effective of the series and P^r the most effective. This effect is clear when the quantities of anthocyanin pigments produced in each genotype are determined, and the relation of the alleles at the *p* locus to flavonol synthesis can be assessed by taking advantage of certain features of the ultraviolet absorption by this group of compounds.

METHODS

The four lateral petals from each of two freshly opened flowers on the same plant were collected and immediately extracted with small (2–3 ml) portions of hot 95 percent ethanol containing 0.4 percent HCl (w/v). The extraction was repeated 5–6 times or until the yield was negligible and the petal residue was essentially colorless. The extracts were pooled and made to either 10 or 25 ml with the extracting solvent in volumetric flasks, the degree of dilution depending on the intensity of pigmentation of the original flowers. A further dilution was frequently necessary to bring absorbance within the range of the spectrophotometer.

Absorption spectra in the visible range were obtained with the Bausch and

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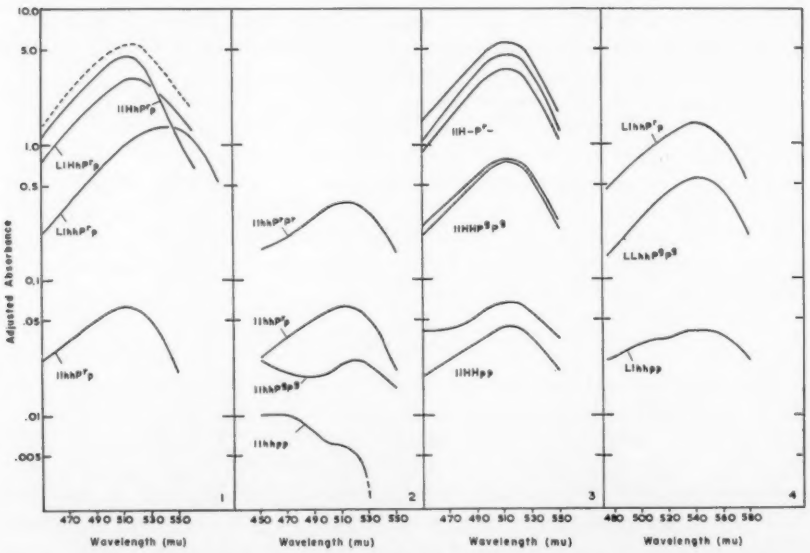
Lomb "Spectronic 20". Ultraviolet spectra were measured with the Beckman Model DU spectrophometer. Where chelation of flavonols was desired, one ml of two percent aluminum chloride prepared in the extraction solvent was added to three ml of extract. On the assumption of a linear relation between absorbance and dilution, each absorbance value has been adjusted to represent the absorbance of an extract of the four lateral petals from one flower in ten ml of extract.

RESULTS

Figure 1 illustrates the interaction of dominant genes in producing the magenta phenotype. The four plants providing these extracts were segregants of a backcross between an inbred white-flowered plant and a heterozygous magenta-flowered plant (*llhhpp* × *LlHhP*^{r}*p*). Consequently all the plants were heterozygous for the genes indicated. *P*^{r}, when other genes are recessive, produces a moderate pigmentation of the petals with pelargonidin derivatives. With *H* present also, the pelargonidin content is 50-fold greater, thus illustrating the synergistic action of these two genes. When *L* is present with *P*^{r}, λ max shifts in correspondence with the replacement of pelargonidin by malvidin, but the total absorbance is almost as great as with red flowers (*llHhP*^{r}*p*). Magenta flowers (*LlHhP*^{r}*p*) exhibit the



FIGURES 1–4.—Absorption in visible wave lengths by extracts of petals of *Impatiens balsamina*. FIGURE 1. Interaction of genes in forming the magenta phenotype. FIGURE 2. Action of genes at the *p* locus in the absence of other dominant pigmentation genes. FIGURE 3. Interaction of alleles at the *p* locus with *H*. FIGURE 4. Interaction of alleles at the *p* locus with *L*. For further explanation see text.

intermediate spectrum expected with a mixture of pelargonidin and malvidin derivatives. If this single example is representative, the total anthocyanin production is somewhat less than the sum of production in *LlhhP^{r}p* and *llHhP^{r}p* as presented in the dotted curve of Figure 1. The shape and position of the magenta curve indicate that the deficiency in total pigment is largely at the expense of pelargonidin rather than malvidin. Other instances in which the synthesis of malvidin seems to have a competitive advantage over the synthesis of pelargonidin have been noted in *Impatiens balsamina* (ALSTON and HAGEN 1958).

Figure 2 illustrates pigment production under the influence of alleles at the *p* locus in the absence of other dominant genes. The lowermost curve is derived from an extract of a white flower and serves to illustrate the low background provided by components under the control of factors other than the genes considered here. The minor absorption at the blue end of the spectrum may account for irregularities in such low curves as the one directly above (*llhhP^{g}P^g*).

The same figure illustrates one of the few effects of heterozygosity which have yet been observed in extracts. The curve designated *llhhP^{r}p* is the higher of two analyses of flowers of the same heterozygous plant, the lower yielding an adjusted absorbance of 0.036 at 512 mu. Three separate homozygous plants of two different cultures and genetic origins gave maximal absorbances between 0.23 and 0.37. The effect of gene dosage can also be observed grossly in faintly colored genotypes involving *L* (*Llhhpp vs. LLhhpp* or *LlhhP^{g}P^g vs. LLhhP^{g}P^g*) and less certainly in those involving *H*, but it cannot be detected in intensely colored flowers.

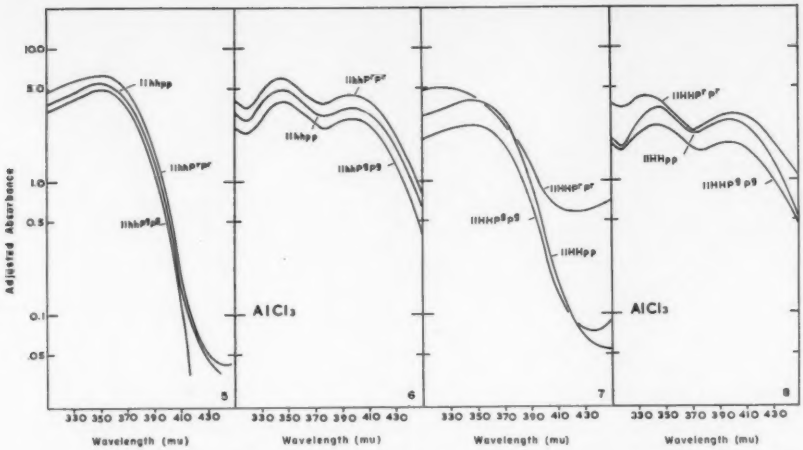
The failure of gene dosage to influence pigmentation of intensely colored flowers is illustrated by the three upper curves of Figure 3. The central curve in this group is derived from a plant of the genotype *llHhP^{r}p*, the uppermost was *llHHP^{r}P^r* and the lowermost was either *llHHP^{r}P^r* or *llHHP^{r}P^g*. Nine analyses of plants including *llHHP^{r}P^r*, *llHHP^{r}P^g*, and *llHhP^{r}p* yielded absorbances at λmax between 2.55 and 5.50, but there was no evident relation between the genotype and the maximal absorption.

The remaining curves of Figure 3 demonstrate the influence of alleles at the *p* locus in regulating total anthocyanin production. *P^g* produces a ten-fold increase in absorbance over *p*, and *P^r* produces at least a five-fold increase over *P^g*. Approximately the same relation is observed in the curves for malvidin derivatives presented as Figure 4.

With the exception of the genotype *llHHP^{r}P^r*, absorption of wave lengths between 300 and 450 mu was remarkably uniform. Each curve exhibited a broad maximum extending from 340 mu to 360 mu (Figures 5 and 7). Flowers with the genotype *llHHP^{r}P^r* consistently yielded greater extinctions at 330 mu and at wave lengths longer than 390 mu.

After addition of aluminum chloride, two peaks appeared with maxima at 345 mu and near 400 mu (Figures 6 and 8). Again the genotype *llHHP^{r}P^r* was an exception with the peak at shorter wave lengths lying at 335 mu.

Absorbances in the ultraviolet range were somewhat greater in extracts of plants recessive for *h* than in those carrying the dominant allele but the differ-



FIGURES 5–8.—Absorption by extracts of petals of *Impatiens balsamina* in ultraviolet wave lengths with and without aluminum chelation. FIGURE 5. Nonchelated extracts from genotypes differing in alleles at the *p* locus. Other pigmentation genes are recessive. FIGURE 6. The same extracts which appear in Figure 5 after adjustment to 0.5 percent aluminum chloride. FIGURE 7. Nonchelated extracts from genotypes carrying *H* but differing in alleles at the *p* locus. FIGURE 8. The same extracts which appear in Figure 7 after adjustment to 0.5 percent aluminum chloride. For further explanation see text.

TABLE 1

Absorbances in ultraviolet wave lengths by chelated and nonchelated extracts of flowers of Impatiens balsamina. *Values are adjusted to the equivalent of four petals from one flower in ten ml of solvent.*

	Absorbance in 95 percent ethanol containing 0.4 percent HCl w/v			Absorbance in 95 percent ethanol containing 0.4 percent HCl w/v and 0.5 percent $AlCl_3$ w/v				
Genotype	No.	Range, 345 mu	Mean, 345 mu	No.	Range, 345 mu	Mean, 345 mu	Range, 400 mu	Mean, 400 mu
lhp	4	4.19–5.47	5.00	3	4.24–4.95	4.68	3.48–3.56	3.51
lhP^g	5	3.66–7.19	5.23	1	4.15	4.15	3.00	3.00
lhP^r	6	4.42–6.38	5.17	2	4.60–6.00	5.30	3.30–4.38	3.84
lHp	4	3.10–4.81	4.11	3	2.83–4.00	3.46	2.13–3.28	2.77
lHP^g	4	2.21–4.91	3.60	2	2.25–2.55	2.40	1.70–1.90	1.80
lHP^r	5	3.35–4.50	3.72	2	2.55–4.18	3.36	1.85–3.20	2.52
–h–	15	3.66–7.19	5.14	6	4.24–6.00	4.80	3.00–4.38	3.54
–H–	13	2.21–4.91	3.80	7	2.25–4.18	3.67	1.70–3.28	2.69

ences were not correlated with the quantities of anthocyanins produced (Table 1).

The flowers providing chelated extracts were collected during December and January. Plants grown at later seasons yielded similar curves from alcoholic extracts but a higher proportion of the absorbing materials was unaffected by aluminum chloride, the long wave peak showing as an inflection point. The

seasonal effect has not been analyzed beyond noting that a period of three weeks on short-day (9 hour) regimen begun after initiation of buds failed to produce "winter-type" absorption in summer grown flowers. Interpretation of this phenomenon is not essential for the purposes of this paper and the absorption by chelated extracts of summer grown plants has been excluded from Table 1.

DISCUSSION

The spectra presented in Figures 1 through 4 represent summed absorption by several glycosides of each anthocyanidin present (ALSTON and HAGEN 1958) and each of these glycosides may have somewhat different spectral characteristics. Consequently no attempt is made to compute pigment content. The curves serve to illustrate the magnitude of influence of each gene and the interaction of genes at different loci. Absorption by other components is apparently negligible in this range.

The genes *H* and P^r have approximately equal effect on pelargonidin production. When present together each supplements the action of the other for the total pigmentation is much greater than the sum of pigmentation produced by separate action. In a similar fashion P^r potentiates the action of *L* in the formation of malvidin derivatives. It seems apparent, therefore, that the *p* locus controls a general reaction in anthocyanin biosynthesis which is independent of the hydroxylation pattern exhibited by the ultimate pigment.

The similarity in structure between anthocyanins and other flavonoid compounds has suggested a common synthetic pathway in early stages (ROBINSON 1936). This assumption is supported by evidence of competition for common precursors between different classes of flavonoids as first noted in dahlia by LAWRENCE and SCOTT-MONCRIEFF (1935) and is also suggested by cases in which a single gene governs substitution in two related nuclear structures. Examples of the latter exist in *Impatiens balsamina* where *L* controls production of the flavonol myricetin and the anthocyanin malvidin and *H* regulates the quantity of pelargonidin derivatives as well as introducing a leucopelargonidin. Competition between flavonols and anthocyanins has not been noted on chromatograms prepared from extracts of balsams.

All genotypes of *Impatiens balsamina* which lack the gene *L* yield petal extracts in which free kaempferol and a single kaempferol glycoside are the only conspicuous flavonols (CLEVENGER 1958). Kaempferol exhibits maximal absorption at 266 mu and 367.5 mu (JURD and GEISSMAN 1956) and its glycosides should absorb at somewhat shorter wave lengths. All flavonoid pigments as well as many simpler phenolic compounds absorb strongly at wave lengths between 250 and 300 mu. As a consequence, extracts of balsam flowers exhibit great optical density in this region. At longer ultraviolet wave lengths, however, flavanones, catechins and leucoanthocyanins contribute little to the total extinction. Among the common flower constituents which have absorption maxima in this region are flavones and flavonols, cinnamic acid derivatives and chlorogenic acid. While these components of balsam petals have not been completely analyzed, it is apparent that

chlorogenic acid is not a significant component. Moreover the cinnamic acids absorb at somewhat shorter wave lengths than the flavones and flavonols (JURD 1956).

Further characterization of the pigments responsible for ultraviolet absorption can be achieved by examining absorption by the metal chelates. JURD and GEISSMAN (1956) have demonstrated that aluminum chelation between the 3-hydroxyl and 4-carbonyl groups produces a large bathochromic shift of the long-wave ultraviolet maximum, in the case of kaempferol from 367.5 mu to 426 mu. Chelation occurs readily at this position in neutral or mildly acid solutions whereas chelation at other susceptible positions, such as 4-carbonyl, 5-hydroxyl or 3′, 4′-dihydroxyl, causes large shifts of absorption maxima only in basic conditions. The major absorption by apigenin, which lacks the 3-hydroxyl group, occurs in ethanol at 326 mu; in ethanol with added aluminum chloride at 342.5 mu.

Glycosidic combination at the 3-position of flavonols might be expected to reduce the bathochromic shift by blocking chelation involving the 3-hydroxyl group. GAGE and WENDER (1949), however, show marked shifts after aluminum chelation of rutin (quercetin-3-rutinoside), xanthorhamnetin (quercetin-3-trirhamnoside) and robinin (kaempferol-3-robinoside-7-rhamnoside). Such combination may provide other chelation sites.

Since kaempferol and its glycoside are prominent constituents of balsam petals and these compounds are susceptible to aluminum chelation, changes in kaempferol concentration should be observable through direct spectrophotometric examination of balsam petal extracts. The curves obtained are precisely those anticipated where absorption by kaempferol and its glycoside is supplemented by the absorption of another component(s) at slightly shorter wave lengths. The other component is apparently incapable of chelation under the conditions of these measurements.

The observations show no action of genes at the *p* locus on flavonol production. Where anthocyanin production can be altered 50-fold by genetic means, absorption due to flavonols remains essentially constant. Because absorption by components which show lesser shift after addition of aluminum also remains constant, other prominent absorbers in this region must also be unaffected by alleles at the *p* locus.

Flavonol production is somewhat less with the gene *H* than in the presence of its recessive allele, but the difference is independent of alleles present at the other loci and also independent of the total quantity of anthocyanin produced. If this gene diverts some intermediate from flavonol synthesis, the intermediate must be partitioned between anthocyanins and other still undetected substances which exist in competitive equilibrium with pelargonidin derivatives.

The shape of the ultraviolet absorption curve is somewhat different in the genotype *llHHP^{r}P^r* than in other genotypes examined. As this is also the genotype which produces the greatest amount of pelargonidin, it is possible that the greater absorption at 330 mu and at wave lengths above 390 mu is caused by derivatives

of pelargonidin itself which has minor absorption peaks at these wave lengths (KARRER 1932).

There is no evidence in *Impatiens balsamina* for competition between anthocyanins and flavonols of the sort existing between anthocyanins and yellow pigments in dahlias (LAWRENCE and SCOTT-MONCRIEFF 1935) or between anthocyanins and aurones in snapdragons (JORGENSEN and GEISSMAN 1955). Moreover the genes which augment anthocyanin production do not provide intermediates which are employed directly for increased flavonol synthesis. Although this information is essential for interpretation of the action of genes which regulate pigmentation in this species, there are still many alternative operations which could be governed by genes at the *p* locus.

SUMMARY

In *Impatiens balsamina*, the allelic genes *p*, P^g and P^r control the quantity of anthocyanins in petals without affecting flavonol production. The gene *H* effects a reduction in kaempferol content which is independent of the amount of pelargonidin produced.

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A SECOND GROUP OF SIMILAR LETHALS IN POPULATIONS OF WILD HOUSE MICE[1]

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THE genotypes found in the natural populations of many animal species regularly include a high frequency of lethal alleles. This fact is most securely established for the species of Drosophila (SPENCER 1947) in several of which it can be estimated that each individual, on the average, is heterozygous for several recessive lethals. MORTON, CROW and MULLER (1956) have pointed out that a similar situation may exist in man. In the one mammalian species in which experimental tests of this point have been made, namely in the house mouse, it has been found that many wild populations include animals heterozygous for a lethal allele at one locus, the only one for which efficient methods of detection have been devised (DUNN 1957).

The question of chief evolutionary interest here is how populations adjust their structures to this high risk of heterozygosity for lethals and other mutations with deleterious effects. The high frequency of lethals at one locus in wild mouse populations provides the opportunity for testing by experimental observations one question relevant to the general problem. This is whether the lethals found in different populations stem from one mutation which then spread by migration, or whether they are due to events occurring independently in the separate populations. In the first case the lethals from different populations should prove to be the same; if the second alternative is true, there is a presumption that mutations occurring at random in different populations should be nonidentical.

Several mutations at this one locus, the complex locus *T* in the mouse, which have occurred under observation in laboratory stocks, have proved to be nonidentical. All were discovered in stocks which already carried a recessive allele, *t*, at this locus. The mutations were thus assumed to represent changes of the type $t^n \rightarrow t^r$. Since t^r proved in each case tested to be a new and different (nonidentical) allele, there appeared to be many sites of mutation, suggesting that the recurrence of the same allele, by chance mutation, would have low probability. The process by which new *t* alleles arise (referred to here as "mutation") will be discussed in a subsequent report. In a few cases it is known to involve recombination in a region near to *T* locus.

However, when lethal alleles derived each from a different wild population were tested for identity, they were found to fall into only two main groups. No differences could be detected, by genetical or embryological criteria, within one

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of these groups consisting of eight lethal *t* alleles each derived from a different population (BENNETT and DUNN 1958). The eight populations were widely separated in the north, south, and west of the United States. While it seemed unlikely that the same allele would arise independently in eight different regions, it was also difficult to suppose that one allele had spread literally all over the country, although in the case of a commensal, this could happen through the agency of man.

In the face of this unexpected finding, it seemed best to suspend judgment until more data on the relations within other such groupings were obtained.

We have now studied the embryological effects of a second group of lethal *t* alleles which by genetical tests are indistinguishable from each other, although each of these alleles is clearly nonidentical with each member of the first group. The group consists of four alleles, three of which were derived from wild populations: t^{w1} (New York 1); t^{w3} (Connecticut 1); and t^{w12} (California 1); the fourth, t^{w20}, was derived under observation in the laboratory from an exception which occurred in the stock of the mutant t^{w15}, a member of group 1 from Kansas. Obviously this set was a significant one to study, because of the inclusion in it of t^{w20}, the first allele observed at origin which did not seem to be different from all others.

The embryological observations to be presented indicate that the four alleles studied have effects so similar that they cannot be distinguished.

MATERIALS AND METHODS

Balanced stocks carrying t^{w1}, t^{w3}, t^{w12}, and t^{w20} were set up by previously described methods (BENNETT and DUNN 1958) and inbred. The four alleles were considered to be on comparable but not isogenic backgrounds. Matings were arranged to provide homozygotes of each of the alleles under study, and all possible combinations between them.

Embryos were recovered from such matings at ages from ten days after fertilization to just before birth. Timing was done by the standard vaginal plug method for younger embryos, and by reference to GRÜNEBERG's (1943) normal stages for older ones. The material reported on in this paper consists of whole mount preparations of embryos of 14 days and older; the study of younger embryos and the detailed histology and morphology of older stages will be published separately.

RESULTS

A total of 126 litters containing 936 embryos were studied at postcopulation stages between 14 days and birth, with the period from 14 to 16 days being most heavily represented. In spite of the rather long period of time covered, the series formed a relatively homogeneous group. In the beginning of the study, all matings were arranged between tailless animals of the genotype T/t^w. This mating system made possible an unequivocal identification of genotype on the basis of tail length, since in such matings three types of embryos are produced:

T/T (dead before 11 days), T/t^{w} (tailless), and t^{w}/t^{w} (normal tailed). It is an interesting developmental point, in view of the interaction of t with T to produce taillessness, that animals carrying two doses of t, either as homozygotes or compound heterozygotes, always have normal tails although they may be otherwise abnormal.

It soon became clear that all normal-tailed embryos found in such matings showed a typical and diagnostic pattern of abnormalities; this held true for both t^{wx}/t^{wx} homozygotes and t^{wx}/t^{wn} compound heterozygotes. Almost all the litters studied contained in addition to recognizable normal-tailed or tailless embryos, some embryos which were being resorbed after death, and which therefore could not be diagnosed as to genotype. All embryos of this type were arbitrarily scored as t^{w}/t^{w}. This method leads to the erroneous inclusion of what is presumably a small number of embryos dying of sporadic causes, and also, in certain matings, of some lethal T/T embryos. The data shown in Table 1 are corrected by omitting from the count those embryos expected to be T/T on the basis of male segregation ratio. Table 1 shows the numbers and condition of abnormal embryos which were found. In each type of mating the frequency of abnormal embryos does not differ significantly from that expected. However, the distribution of abnormal embryos with regard to their time of death does vary considerably from one type of mating to another, and also among individual litters in the same type.

It is clear that the lethal period for embryos carrying two doses of these alleles is an extended one, covering the period from eight or nine days after fertilization to birth, that is, over somewhat more than the last half of gestation. There appear to be two critical periods early in development. The first occurs at about nine

TABLE 1

Numbers and conditions of abnormal embryos

Parental genotypes	No. litters	No. embryos	No.* normal	No.† abn	%‡ abn	No. abn alive	%§ abn alive	No. abn dead	No. moles	%‖ moles
$t^{w1} \times t^{w1}$	15	96	58	38	39.6	13	34.2	7	18	47.3
$t^{w3} \times t^{w3}$	17	106	64	42	39.6	1	2.4	12	29	69.0
$t^{w12} \times t^{w12}$	11	81	37	44	54.2	4	8.9	8	30	68.2
$t^{w20} \times t^{w20}$	13	105	61	45	42.5	0	0	2	43	95.5
$t^{w1} \times t^{w3}$	17	136	64	72	52.3	31	42.4	20	21	29.2
$t^{w1} \times t^{w12}$	12	79	45	34	43.0	15	44.1	6	13	38.1
$t^{w1} \times t^{w20}$	14	123	70	53	43.0	7	13.2	14	32	60.4
$t^{w3} \times t^{w12}$	9	68	30	38	55.8	2	5.3	6	30	79.0
$t^{w3} \times t^{w20}$	7	58	25	33	56.8	3	9.1	4	26	78.7
$t^{w12} \times t^{w20}$	11	95	46	49	51.6	2	4.4	6	41	82.7

* Number without obvious abnormalities.
† Number recognizably abnormal.
‡ $\frac{\text{No. abnormal}}{\text{Total embryos}} \times 100.$
§ $\frac{\text{No. abnormal and alive}}{\text{Total abnormal}} \times 100.$
‖ $\frac{\text{No. moles}}{\text{Total abnormal}} \times 100.$

days; the victims of this are seen only as completely macerated "moles" when litters of the age under discussion here are examined. The second is around 12 days, and the embryos dying at this time remain identifiable, although macerated, for at least four or five days. Figure 1 shows the estimated time of death for

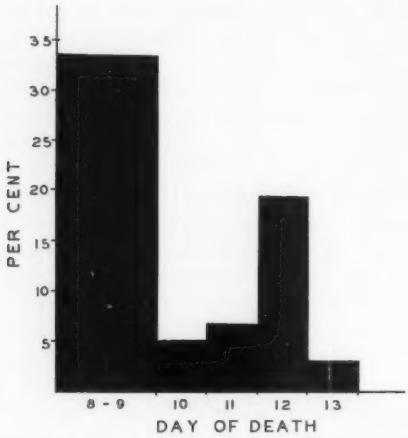

FIGURE 1.—Distribution, according to day of death, of dead embryos from litters of 14–17 days gestation. A total of 268 embryos are represented.

embryos found dead in litters dissected at postcopulation ages of 14–16 days. The deaths of embryos which survive early critical periods appear to be distributed at random in the succeeding days.

Table 1 shows clearly that the proportion of embryos succumbing in the critical periods varies quite markedly from one allele to another. For instance, about 95 percent of embryos homozygous for t^{w20} die early and are represented by moles, as opposed to 47 percent for t^{w1}. A difference of this magnitude would seem almost enough evidence to categorize t^{w1} and t^{w20} as different alleles, if it were not for the fact that the homozygotes for t^{w3} and t^{w12} fall in intermediate positions, thus giving the impression of a continuous spectrum. Furthermore, some compounds of t^{w20} with each of the other alleles do survive the early critical periods. These present a syndrome of abnormalities identical with that found in any of the homozygotes. This points to the probability that the four alleles act in similar ways. If this is so, the differences in expression would have to be ascribed to dissimilarities in the genetic background of the four stocks. The relative length of survival of lethal embryos from the other group of alleles previously reported was ascribed to variation in content of genetic modifiers (BENNETT and DUNN 1958).

The abnormal embryos which were recovered alive from all four stocks presented an external appearance that was typically uniform, regardless of genotype. A tail of normal length was present in all of them. All were considerably

reduced in size, ranging from one half to three fourths of normal length. Microcephaly was constantly present, although variable in degree. In all, the bony cranial vault was abnormally open and the cerebral hemispheres protruded through it, giving the head a very typical peaked appearance. Generalized subcutaneous edema was a constant feature in all embryos studied at 14 days and older, although its degree varied somewhat. Several other abnormalities which were probably consequences of the edema were also frequently present. These included enlarged pericardial cavity, smaller hair follicles, open eyelids, and retardation of the closing of the external ear by the pinna. Placentae of abnormal embryos seemed grossly normal; they were smaller than normal, but in proportion to the size of the embryos.

Studies of cleared whole mount preparations show that all of the abnormal embryos of 14 days and later which were recognized by the external criteria described above have a rather constant complement of major skeletal abnormalities. Both cartilageneous and membrane bones are affected. The bony abnormalities fall into several categories: *(1) Absence of ossification in the skull:* In almost all embryos studied, there is no sign of ossification of the supraoccipital, or presphenoid, or the body of the basisphenoid. The failure of these bones to ossify is correlated with a shortening of the skull in an anterior-posterior direction. Failure of ossification was not noted in other bones. *(2) Fusion:* In embryos of 16 days and older, the frontal and parietal bones were always completely fused, with no evidence whatever of separate ossification centers. In younger embryos there is occasionally evidence that two centers were present initially, but not maintained for long. Furthermore, the fused fronto-parietals on each side of the head show little tendency to approach each other, thus leaving the typical large oval opening in the cranial vault through which the cerebral hemispheres protrude. Several other bony fusions exist in the base of the skull; these seem also to be connected with a reduced amount of space in the cranial cavity. *(3) Deformed or incomplete bones:* There are numerous minor abnormalities of shape and structure in bones throughout the body, that is, scapula, humerus, mandible. No common process (such as retardation) can be identified as responsible for this. *(4) General faulty histogenesis of bone:* Bone throughout the body seems to be generally thin, uneven and irregular, as compared to normal developing bone.

DISCUSSION

The most important conclusion from the above observations is that abnormal embryos diagnosed as homozygous $t^{w_{5x}}/t^{w_{5x}}$ from all four stocks show a common though variable syndrome of developmental abnormalities. This syndrome is also characteristic of the compounds $t^{w_{5x}}/t^{w_{5n}}$ containing alleles from each of two different stocks. It is significant that this syndrome includes a pattern of abnormalities not hitherto observed as effects of the other lethal alleles which have been studied at this locus. The resemblance in these unusual features of all the homozygotes and compounds suggests that the course of development in all of them has been altered by some common cause. This would be the case if the four alleles were

similar or identical, and we shall adopt this hypothesis for the purpose of the ensuing discussion.

If the alleles found in three widely separated populations are the same, how shall we explain the geographic distribution of animals carrying them? They could represent descendants of one mutation which occurred in a population ancestral to all three. In that case the most probable means of dispersal would be transport by man. The alleles could, on the other hand, have arisen as independent mutations. The probability of this would have to be judged by the frequency with which identical mutants recur. On the evidence of mutants observed at origin in laboratory stocks we have heretofore judged this probability to be low, and have considered the locus as consisting of so many sites of mutation that random occurrence of two changes at the same site would be a rare event.

Now, however, we have observed for the first time that one mutant, t^{w20}, which is indistinguishable embryologically or genetically from a previously known group of alleles, has arisen independently. This requires some reconsideration of the structure of the locus.

It has been assumed that some lethal *t* mutants at the complex *T* locus are connected with inversions, since of the lethals studied three (t^0, t^1, t^{12}) prevent crossing over in the region of *T* and markers rather closely linked to it. It has been further assumed that the considerable instability of mutant *t* alleles (as demonstrated by the production, in the presence of *t*, of new "mutants" with a frequency of about one in 450) may be the result of either, or both, of two processes: (1) partial reinversion of the aberrant chromosome segment, and (2) production of new point mutations or of recombinants within an area already made unstable by the presence of an inversion (DUNN and GLUECKSOHN-SCHOENHEIMER 1950).

The fact that many of the *t* mutants observed at or near origin in the laboratory are nonidentical would suggest that the number of "sites of mutation" is very high, hence that the region affected is large and complex. Thus, the tendency has been to consider that either of the two types of events mentioned above (which would result in the production of a new *t* mutant) would probably be unique and generally nonrepeatable, because of the length and complexity of the chromosomal segment in which it occurred. Under this hypothesis, it would be expected, therefore, that each new *t* mutant arising under observation in the laboratory would be different from any others previously found. It might also be expected that, by and large, mutants recovered from geographically widely separated populations would be different. A certain proportion of alleles extracted from wild populations of course would be expected to be the same, because of the migrations or transportation of mice carrying *t* alleles from one population to another. It is logical to assume that such processes will occur, and that, because of the high segregation ratio found in males, an introduced *t* allele will tend to spread in new populations. Unfortunately, no definitive data are available for making an estimate of the importance of this factor.

The evidence which is discussed in this paper does not appear to bear out the hypothesis of uniqueness of new *t* mutants. Thus t^{w20}, a mutant which arose under

observation in the laboratory, appears operationally to be not unique, but closely similar or identical to three other mutants derived from separate wild populations.

Additional evidence of the same sort comes from several other sources:

(1) The study of t^{w5}, t^{w6}, t^{w11}, t^{w13}, t^{w14}, t^{w15}, t^{w16}, t^{w17}, which were derived from wild populations in, respectively, New York, Florida, Connecticut, Texas, Vermont, Kansas, Vermont, and Arizona. All of these alleles have been found to be nondistinguishable by genetic cross test; they produce lethal homozygotes and lethal compound heterozygotes that are indistinguishable from one another (BENNETT and DUNN 1958 and unpublished).

(2) The comparison of t^9 (discovered in France) and t^{w4} (from Wisconsin) which indicates that they are not different (DUNN and SUCKLING 1956).

(3) The comparison of t^9 and t^6 (Edinburgh) which indicates that they cannot be distinguished genetically (DUNN and GLUECKSOHN-WAELSCH 1950).

Thus, to sum up, there is evidence that in one case an allele which arose under observation is not different from other previously known alleles. In several other cases, the geographical separation between similar alleles is so extensive as to make it reasonable to assume independent similar acts of mutation for their origin, rather than transportation across such great distances.

It seems, therefore, that this complex locus which encompasses the possibility for many different inversions or sites of mutation, may possess also a certain regularity of structure which makes possible the precise repetition of certain mutational events.

This sort of speculation about the physical nature of the *T* locus obtains support, in a formal way, from studies on Drosophila. We know for instance that crossover breaks are not necessarily distributed at random over the length of the chromosomes, but that certain areas seem more vulnerable to that process than others. Another case in point is Bar. The Bar "mutation" is essentially a duplication of a section of several bands visible in the salivary chromosome. Another form, ultra-Bar, consists of three repeated sections. Changes from Bar to ultra-Bar and normal, and back again, occur relatively frequently (one in 1500), being brought about by crossing over between the duplicated sections. Moreover, and this is the important point in this context, crossing over appears to occur only between Bar segments, and not within them, thus preserving the integrity of the section (BRIDGES 1936). However, it should also be pointed out that the Bar "mutation" has occurred only once and not repeatedly as has been the case with the *t* alleles. Another formal comparison can be made with the roughest[3] inversion which, in a stock studied by GRÜNEBERG (1937) was found to have reinverted completely, apparently by breaks occurring at the same points by which the original inversion had been produced.

This sort of evidence may make it justifiable to consider the *T* locus as consisting of a number of units which have a relative physical integrity. Therefore, it is not surprising that at least two of the mutants arising at this locus appear to be repeat mutations of independent origin.

SUMMARY

A group of four genetically indistinguishable lethal alleles at locus *T* in the house mouse, three (t^{w1}, t^{w3}, t^{w12}) derived each from a different wild population and one (t^{w20}) derived in the laboratory by mutation from another *t* allele, have proved to be also embryologically indistinguishable. Embryos homozygous or compound heterozygous for any of these alleles show definite diagnostic abnormalities and are not viable; their deaths are distributed over the last day of gestation, with lethal crises at eight or nine days and 12 days. Since neither genetic nor embryological operational tests can distinguish among these alleles, the conclusion is that they are either closely similar or identical. Thus, t^{w20} is the first *t* mutant actually observed at origin which is not clearly different from all others. The significance of this finding for our thinking about the structure and behavior of the *T* locus is discussed.

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STUDIES ON THE IMMOBILIZATION ANTIGENS OF PARAMECIUM. IV. PROPERTIES OF THE DIFFERENT ANTIGENS[1]

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EACH stock, or strain, of Paramecium can produce a number of antigenic types or serotypes. Each serotype is immobilized by its homologous antiserum, but not by antiserum made against other serotypes. The complex cytoplasmic, genic, and environmental factors that determine which of the possible serotypes is manifested in a given animal were discovered by SONNEBORN (1948). The inheritance of serotypes in Paramecium has been reviewed recently by BEALE (1957). Animals of each serotype contain a single characteristic protein or immobilization antigen which is localized primarily in the cilia and body wall. The specificity of most, if not all, of the immobilization antigens is determined by the alleles at several different loci—one locus being responsible for the specificity of each serotype. Since individual cells generally show only one serotype at a time, only one genetic locus comes to expression at a time. Within a stock serotypic differences are determined either by the environment or by cytoplasmic inheritance. In the first paper of this series (PREER 1959a) it was shown how the immobilization antigens can be followed quantitatively *in vitro* using antigen-antibody precipitation in agar gel. PREER (1959b) has recently shown how the antigens can be isolated chemically, and finally PREER (1959c) gave an account of the properties of antigen A of stock 51. It was found that the A antigen is a large asymmetric protein molecule with a molecular weight of around a quarter of a million. The present and last paper of the series deals with a comparative study of several of the immobilization antigens of syngens (varieties) 2 and 4 of *Paramecium aurelia*. Serology, solubility in ammonium sulfate, and, to a lesser extent, sensitivity to proteolytic enzymes have been studied.

MATERIALS AND METHODS

Stocks of *Paramecium aurelia*, culture methods, the method of isolating the immobilization antigens, and serological methods have already been described (PREER 1959a; PREER and PREER 1959). A few points, as well as some special methods, require comment.

Each stock of *P. aurelia* exhibits a series of serotypes designated A, B, C, etc. When the serotypes of different stocks are compared, many are found to be

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indistinguishable or closely related. Within a syngen, all homologous serotypes are designated by the same letter. Thus 51 A of syngen 4 (stock 51, serotype A) is closely related to 29 A of syngen 4 and 51 B is indistinguishable from 29 B. The serotypes in each syngen, however, have been given letter designations somewhat independently of each other, and corresponding letters indicate homologies only in certain cases. We have utilized serotypes A, B, C, D, E, G, H, J, M, and Q of syngen 4, and also serotypes E and G of syngen 2. The two E serotypes are completely unrelated (do not cross react) while the two G serotypes are closely related. Since most of the work has been done on syngen 4, the letter designations used in this paper refer to syngen 4, unless otherwise indicated.

In the serological technique of double diffusion in agar used here (PREER 1956), a layer of agar is established in a small tube with a layer of serum below the agar and a layer of antigen above. Where the diffusing antigens and antibodies meet within the agar, bands of precipitation are formed. The position of a given band is dependent upon the relative diffusion coefficients and relative concentrations of antigen and antibody. Since these are rarely the same for independent antigen-antibody systems, each antigen usually forms a separate band.

The bands of precipitation formed when both heterologous and homologous cross-reacting antigens are present in the antigen layer present a special problem. In general, it can be expected that if the homologous antigen is sufficiently in excess, it will absorb all the antibodies, and the heterologous antigen cannot form a band of precipitation behind (nearest the antigen-agar interface) the band produced by the homologous antigen. On the other hand, if the heterologous antigen is present in sufficient excess two bands of precipitation will be formed. The first (farthest from the antigen-agar interface) will be produced by the reaction of the heterologous antigen with those antibodies with which it can combine, and the second will be produced by the reaction of the homologous antigen with the remaining antibody.

The determinations of ammonium sulfate solubility were made as described earlier (PREER 1959b, c). Animals of the appropriate serotype were extracted in four volumes of one part 30 percent alcohol plus one part of buffered saline (0.15 M sodium chloride in 0.01 M sodium phosphate buffer, pH 7.0) for one hour at 2°C. The pH was reduced to 2.0 with 0.2 N HCl and the precipitate removed by centrifugation at 25,000 G for three minutes. The pH was raised to 7.0 with 0.2 N NaOH and the precipitate again removed and discarded. Fractional precipitation with ammonium sulfate was then carried out as follows. Sufficient saturated (23°C) ammonium sulfate (pH 7.5) to reach the first desired level of saturation was added. The precipitate was removed by centrifugation, washed in 75 percent saturated ammonium sulfate, and redissolved in buffered saline. The supernatant was then raised to a higher level of saturation by adding more saturated ammonium sulfate, and the process continued until the desired final level of saturation was attained.

Immobilization antigen was determined in each fraction quantitatively by gel diffusion according to the procedure described earlier (PREER 1959a). The

method is based upon the fact that, for constant antibody concentration, the position of the band (measured as the distance from the antigen-agar interface to the band, divided by the total agar length) is linearly related to the logarithm of the antigen concentration. An estimate of total protein was obtained spectrophotometrically using the method of WARBURG and CHRISTIAN (1941). Values obtained by the method of WARBURG and CHRISTIAN were multiplied by the factor 0.85 as recommended by PREER (1959c).

Tests for the action of proteolytic enzymes were made by exposing either crude extract or purified antigen to the enzymes. After a suitable incubation period, the mixture was added to antiserum, and then paramecia were added to see if the antigen had absorbed antibody from the serum or whether its capacity to react with antibody had been destroyed by the enzyme. The detailed procedure, including appropriate controls, was as follows. Crystalline trypsin (Nutritional Biochemicals Co.) was dissolved in 0.01 M tris (hydroxymethyl) aminomethane buffer, pH 7.6, containing 0.04 M $CaCl_2$. Four preparations were made in performing a test: (1) Two parts buffer (2) One part enzyme, one part buffer (3) One part antigen (containing about 300–400 micrograms per milliliter), one part buffer (4) One part enzyme, one part antigen. Each of the four preparations was incubated two to three hours at 37°C, and then to each was added two parts of antiserum diluted with buffered saline. The antiserum was 25 times as strong as the greatest dilution giving complete immobilization in one half hour. After five minutes the mixtures were diluted by adding 36 parts of ten percent Ringer's solution and ten parts of a culture containing primarily animals of the serotype being studied (homologous) as well as a few animals of a non-homologous serotype. After one half hour the animals were observed under the microscope. In all mixtures the non-homologous animals should be unaffected, showing that the constituents are nontoxic. In preparation (1) the homologous animals should be immobilized, demonstrating active specific antibodies. In mixture (2) animals should be immobilized showing that the enzyme has no harmful effect on the antibodies. In preparation (3) the animals should be unaffected, demonstrating the activity of the unimpaired antigen in absorbing antibodies. In preparation (4) the homologous animals are immobilized if the enzyme has destroyed the antigen, thereby leaving the antibodies to react with the animals, and unaffected if the antigen has not been destroyed by the enzyme.

Crystalline chymotrypsin (salt free, Nutritional Biochemicals Co.) was dissolved in 0.1 N sodium phosphate buffer, pH 7.6, and tests were conducted in the same way as those on trypsin.

Crystalline pepsin (Nutritional Biochemicals Co.) was dissolved in 0.06 N HCl. The antigen preparations were adjusted to pH 2.0 with 0.2 N HCl. The usual four preparations (see above) were made, using 0.06 N HCl in place of buffer, and incubated in the usual way. Then each of the preparations was neutralized by adding one part of 0.2 M Na_2HPO_4 to two parts of each mixture. Serum, ten percent Ringer's solution, and animals were then added in appropriate amounts taking into account the dilution introduced by adding the Na_2HPO_4.

RESULTS

Serology

Purified immobilization antigens in buffered saline were adjusted to a concentration of 85 micrograms per milliliter and double diffusion tubes were set against both homologous and heterologous sera. Some of the tubes are shown in Figure 1.

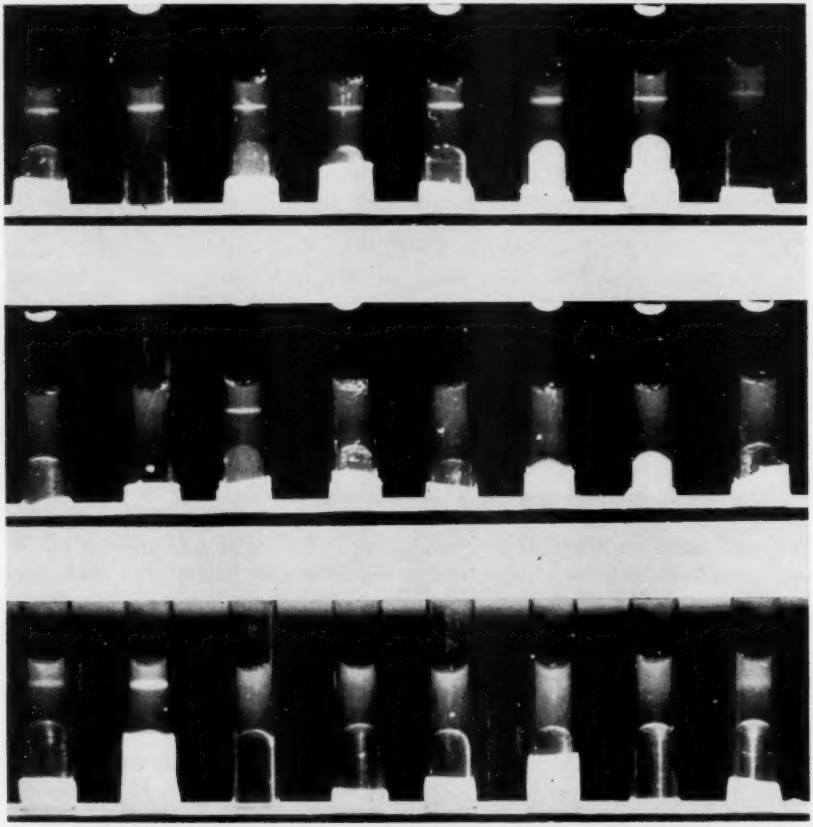

FIGURE 1.—Reactions of purified antigens with various sera. The top layer in each tube is the antigen solution; the middle layer is agar and contains the precipitation zones; the bottom layer is serum; a paraffin plug is visible at the bottom of some of the tubes. The eight tubes in each figure were set with eight sera prepared against whole homogenates; the same eight sera were used in each set; reading from left to right, they were (1) 51 A, (2) 51 B, (3) 51 C, (4) 51 D, (5) 51 E, (6) 51 H, (7) 131 M, (8) syngen 2 E. a. Homologous reactions; each tube set with the corresponding antigen. b. Complete specificity of the C antigen which was used in all tubes. c. The B antigen was used in all tubes and cross-reacts only with the A and syngen 2 E sera in tubes (1) and (8).

Figure 1a shows homologous combinations. Since the sera were prepared against homogenized whole animals, antibodies against many nonimmobilization antigens were present. But since the antigen preparations were purified, only a single band of precipitation is found in each tube. Figure 1b shows the 51 C (stock 51, serotype C) antigen set against the same series of sera. 51 C is completely specific and reacts only with its homologous antiserum. Figure 1c shows 51 B set against the sera. In this case, in addition to the strong homologous reaction, somewhat weaker bands are found with the 51 A and 4 E (syngen 2) sera. It will be shown presently that these weak reactions are true cross reactions caused by the reaction of the B antigen with antibodies against other serotype antigens. In Table 1 the results of setting the antigens with a number of sera are

TABLE 1

Reaction of purified immobilization antigens with various sera in gel diffusion. Twenty-one sera (designated by number in the first row of the table) prepared against the indicated serotypes were diffused against each purified antigen. + indicates a single band of precipitation; no entry indicates no reaction

	Sera																					
	C		E		H		D		J		M		A		B		Q	syn. 2 E		G		
Antigens	24	64	17	54	58	61	74	123	75	78	43	81	56	110	45	119	117	132	149	116	28	
C	+	+			+																	
E			+	+																		
H					+	+	+															
D							+	+	+	+	+	+										
J		+					+	+	+	+	+	+	+		+						+	
M							+	+	+	+	+	+										
A													+	+	+	+	+	+	+	+		
B	+		+										+	+	+	+	+	+	+	+	+	
syn. 2 E													+	+	+	+	+	+	+	+	+	

given. All sera were from the collection of PROF. T. M. SONNEBORN except #132 and #149 which were prepared by the author. It is noted that each antigen (except Q) was set against two sera prepared against each serotype, and that all combinations were made. In the table, a + indicates that a band was obtained; no designation means that no band was found. Homologous bands were invariably more intense than heterologous bands. Inspection of the table shows that, on the basis of the pattern of cross reaction, D, J, and M form one group, and A, B, Q, syngen 2 E, and G form another, with C, E, and H separate from each other and the groups. The arrangement of serotypes in the table has been made so as to emphasize these relations. The evidence indicates that these groups consist of serologically related antigens. A few cross reactions appear outside the groups sporadically, but they are not sufficiently frequent to disturb the general scheme of grouping.

Cross reactions can also be seen in immobilization tests, i.e., when animals are treated with diluted antiserum. Table 2 contains the results of immobilization tests on the same serotypes and sera given in Table 1. Immobilization tests were

TABLE 2

Reaction of living paramecia with various sera in immobilization tests. Sera (same as those in Table 1) were tested against paramecia of each serotype. + indicates immobilization or strong retardation of paramecia in a dilution of 1/12.5 serum after 2 hours at 27°C; no entry indicates no reaction

Serotypes of paramecia	Sera																							
	C		E		H		D		J		M		A		B		Q	syn. 2 E		G				
	24	64	17	54	58	61	74	123	75	78	43	81	56	110	45	119	117	132	149	116	28			
C	+	+					+					+									+			
E			+	+	+										+									
H					+	+						+												
D							+	+		+		+							+				
J							+	+	+	+	+	+												
M							+	+		+	+	+												
A			+								+		+	+	+					+	+			
B	+		+								+		+	+	+	+	+		+	+	+			
syn. 2 E													+	+				+	+	+				

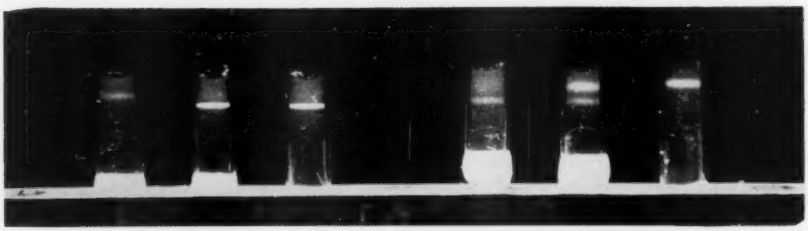

FIGURE 2.—Serological analysis of the cross reaction between A and B. All six tubes are set with B serum. Tube (1) is set with a low concentration of A antigen; tube (3) has a high concentration of B; tube (2) has a mixture of the two, and contains only one band showing all the antibodies can react with the homologous antigen. Tube (4) has a high concentration of A; tube (6) has a low concentration of B. Tube (5) contains the mixture and shows two bands, revealing that some of the antibodies may react with B.

performed as prescribed by SONNEBORN (1950) and a + in the table indicates that animals were either immobilized or markedly retarded in their swimming by the serum in concentrations of 1/12.5 or less. It is noted that the cross reactions are not all the same as observed in the gel diffusion tests, but that the same general pattern is followed.

The cross reaction between 51 A and 51 B was chosen for special study. Each gives a strong band with homologous serum and a weak band with heterologous. Information about the nature of the cross reaction is obtained by setting mixtures of the A and B antigens against the sera. Figure 2 shows the results of the tests on serum S#45 (51 B). Tube (1) shows the relatively weak band produced by using 47 micrograms per milliliter of the heterologous 51 A antigen. Tube (3) was set with 425 micrograms per milliliter of homologous 51 B antigen and gives a strong band lower in position. Tube (2) was set with a mixture containing 47

micrograms of A and 425 micrograms of B per milliliter. Only one band is obtained, showing that the concentrated B antigen has absorbed the antibodies from the serum before it could reach the more dilute A.

When the ratio of A to B is reversed, a very different result is obtained. The antigen layer of tube (4) contains 375 micrograms of A per milliliter and forms a weak heterologous band near the center of the agar. Tube (6) contains 95 micrograms of B per milliliter and forms an intense homologous band just within the agar. Tube (5) contains 375 micrograms of A per milliliter and 95 micrograms of B per milliliter and two bands appear. The heterologous A band forms first, reacting with some of the antibody, and the remaining antibody, being unable to react with the heterologous A, diffuses further into the agar and reacts with B to form the characteristically intense homologous band. All these results were obtained with anti-B serum. When the experiment was repeated with the anti-51 A serum S#110, analogous results were obtained. One band is obtained in mixtures in which the homologous antigen is in excess, two bands are obtained when the heterologous antigen is in excess. These experiments show that sera S#45 (B) and S#110 (A) have some antibodies which can react with the heterologous as well as the homologous antigens, but that all antibody can react with the homologous antigens. The data provide no evidence that cross reactions are the result of small amounts of heterologous antigen present in the extracts of each serotype. If the extracts from each serotype contained mixtures of antigens, more than one band should be seen, but they are not. Instead, some of the antibodies formed in response to one antigen can also react with closely related antigens.

Solubility

Solubility of the antigens in ammonium sulfate was determined in one experiment by measuring the amount of antigen precipitated from acid treated extracts (see above). Ammonium sulfate was added until the solution reached 38 percent. saturation. The precipitate was removed, redissolved, and assayed for immobilization antigen (using gel diffusion) and protein (spectrophotometrically). The ammonium sulfate concentration was now increased stepwise to 47 percent, 56 percent, 65 percent, and 74 percent, the precipitates being assayed for antigen and protein at each step. One half to two milliliters of packed cells were used for each extraction, and fractionations of antigen from each serotype were done in duplicate on separate cultures of animals. All serotypes within a stock were recently derived from the progeny of one animal. The results are shown graphically in Figure 3. The determinations of the amounts of antigen and protein in each fraction are given in percent of the total in all five fractions. It is noted that the data on antigen are very closely paralleled in every case by the data on protein. There is a tendency for the protein to exceed the antigen in fractions in which there is only little antigen (particularly in the higher ammonium sulfate concentrations), and a corresponding tendency for the measure of antigen to exceed protein where antigen is high. This discrepancy would be expected if the major protein were the antigen, and if a small amount of contaminating protein is also present.

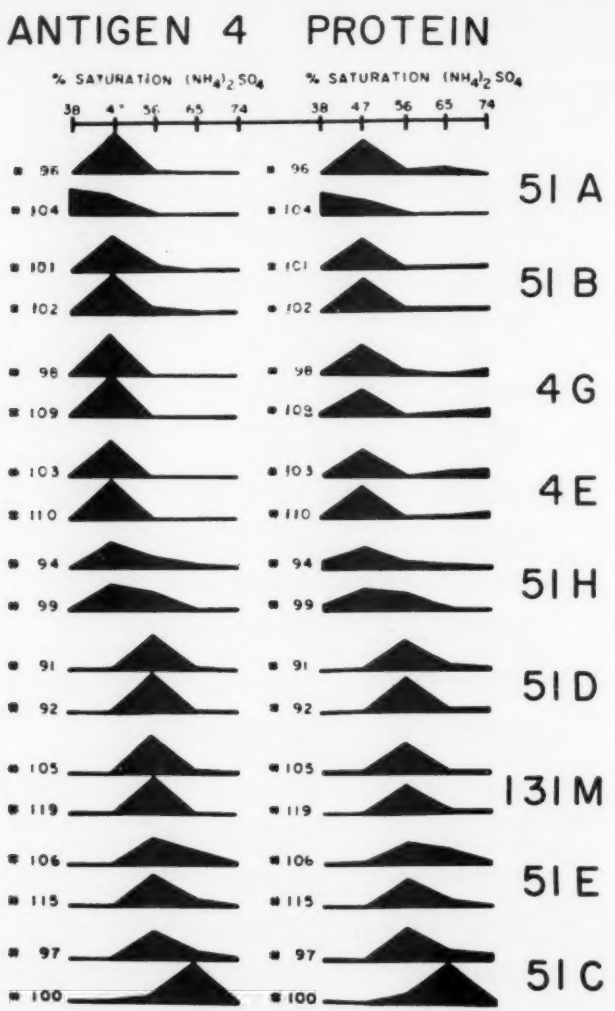

FIGURE 3.—Ammonium sulfate solubility of immobilization antigens of different serotypes in partially purified extracts. The percent precipitated by increasing the salt concentration to the indicated percent levels of saturation was obtained on two antigen preparations from different serotypes. Measurements on antigen 4 (the immobilization antigen) by gel diffusion are given in the left hand column, while measurements based on total protein measured spectrophotometrically are given in the right hand column.

It should be noted that the parallel between antigen and protein is maintained even when duplicate runs do not agree, and even when different antigens with different solubilities are studied.

The data of Figure 3 also show that the solubilities of the different antigens are different. The discrepancy between duplicate runs seen in certain cases results from the very great differences in antigen solubility in ammonium sulfate solutions of only slightly different concentrations. A very steep curve results when solubility is plotted against ammonium sulfate concentration (See PREER 1959c for a solubility curve for antigen 51 A). As a result, great differences in duplicate runs can be seen if one of the five levels of ammonium sulfate concentration used here happens to be critical for a given antigen. 51 A is a good example, for in the antigen concentrations used in the experiments, 38 percent saturation is critical. A very small change in solubility or in ammonium sulfate concentration can result in the precipitation of virtually all of the antigen, or in most of it remaining in solution. It is seen that 51 A, 51 B, 4 G (syngen 2), and 4 E (syngen 2) show the lowest solubilities. 51 H is slightly higher, 51 D and 131 M are next highest, and 51 C and 51 E are still higher. Extensive data other than those shown in Figure 3 on the solubility of immobilization antigen from cilia, and on both crude and purified antigen from whole homogenates, confirm and extend these conclusions. Four and possibly five solubility groups are found. (1) 51 A, 51 B, 51 Q, 4 G (syngen 2), and 4 E (syngen 2) constitute one group; (2) 51 H is slightly, but significantly different; (3) 51 D, 29 J, and 131 M constitute another group; (4) 51 C and 51 E are the most soluble, and there are indications that 51 C is slightly more soluble than 51 E. The data given above on cross reactions parallel these findings remarkably well. The serotypes in group (1) are all serologically related; those in group (3) are also related; while 51 H, 51 C and 51 E are unrelated.

Sensitivity to proteolytic enzymes

The method of conducting the tests is described above. Antigen was assayed by its ability to absorb or block immobilization antibody and thereby prevent the immobilization of paramecia. Enzymatic activity was demonstrated if the enzyme was able to prevent antigen from reacting with immobilizing antibody. Appropriate controls indicated that the sera did contain active immobilizing antibodies, that the unimpaired antigen was able to absorb antibodies, that the enzymes did not destroy the antibodies, and that no substances injurious to the living animals (other than specific antibody) were present in the test. Controls were satisfactory even when the enzymes were used in concentrations as high as 0.2 percent in the incubation mixtures.

In one experiment 51 D antigen #35 (237 micrograms per milliliter, purified from a homogenate after overnight extraction at 2°C) was completely inactivated in three hours in 0.2 percent trypsin, and partially inactivated in 0.05 percent trypsin. 0.2 percent trypsin was able to destroy 51 D in numerous other experiments. Inactivation (usually complete) was obtained not only in purified antigen,

but in antigen in ciliary extracts, and in crude extracts of whole homogenates, either freshly prepared, or after overnight extraction. The 51 D antigen #35 was completely destroyed in concentrations as low as 0.05 percent chymotrypsin, and was partially inactivated in 0.01 percent. Chymotrypsin proved very active on various other antigen preparations. Pepsin was even more active than chymotrypsin; complete inactivation of antigen #35 was obtained in 0.0015 percent pepsin, and partial inactivation at even lower concentrations. Other purified and crude antigen extracts were tested in pepsin concentrations of 0.05–0.01 percent pepsin and were completely inactivated. Tests showed that pepsin acted very quickly, the stronger concentrations destroying the antigen in minutes.

Antigen 51 A was also inactivated by chymotrypsin and pepsin. However, unlike 51 D, it was not inactivated by trypsin even in the highest concentrations used (0.2 percent). The resistance to trypsin was observed in purified preparations as well as in fresh extracts of homogenates. Other serotypes were not tested.

DISCUSSION

The serological analysis of the cross reaction between the 51 A and 51 B antigens reveals that although all of the immobilization antibodies in each serum can react with their homologous antigen, a portion can also react with the heterologous antigen. BALBINDER and PREER (1959) reached the same conclusion in a study of the cross reacting serotypes G and E of stock 28, syngen 2. The other cases of cross reactions were not similarly tested. Nevertheless, since no more than one band was produced in any gel diffusion tube, it is probable that the remaining cross reactions are of the type analyzed, rather than being caused by a mixture of immobilization antigens. Consequently the reaction of antibody against one antigen with a heterologous antigen is evidence of structural similarity of the molecules. Antigens may thus be placed into related groups: (1) A, B, G, Q, and syngen 2 E; (2) D, J, and M. The other antigens studied, C, E, H, are unrelated. The exceptional cross reactions in gel diffusion may be ascribed to a more remote relationship, and to the variability of rabbits in responding to similar molecules. Discrepancies between the cross reactions of gel diffusion and those of immobilization tests are not surprising in view of the fact that the activity of serum is only partly correlated with its activity in immobilization (FINGER 1956). The explanation for this fact is unknown, but possibly is explained by differing efficiencies of antibody in these two activities. This same phenomenon may also explain the greater variability of immobilization cross reactions as compared to gel diffusion cross reactions. The possibility of immobilization reactions dependent on antigens other than the usual (antigen 4) family of immobilization antigens is perhaps worth consideration. The only other soluble ciliary antigen known, however, is antigen 5 (PREER 1959b), and a serum (P#183) against purified antigen 5 does not immobilize.

The grouping of antigens given here can be supported on other grounds. D and M in some cases (MARGOLIN 1956) can coexist in single animals, constituting an exception to the rule that the different serotypes found in homozygous lines are

mutually exclusive. Somewhat related to this situation is the relative ease of transformation of one type into another. 51 H when brought from 12°C to 27° often transforms to D and J and often to D through J (SCHNELLER, personal communication). Serotypes G and E of syngen 2 fall into the same cross reacting group and are easily transformed one into the other. AUSTIN *et al.* (1956 and personal communication) report that when various serotypes are treated with the antibiotic patulin and other agents, transformation is to B, A, N, and Q—all cross-reacting types. Such facts must be interpreted with caution, however, for *all* types in stock 51 go to A at high temperatures. Furthermore within one related group, A and syngen 2 E are more stable at high temperatures, while B and G are stable at low temperatures.

The solubilities of the antigens in ammonium sulfate parallel the groupings based on cross reactions remarkably well. The relation between A, B, Q, G, and syngen 2 E is confirmed, as well as the relation between D, J, and M. Even the fact that H, C, and E are unrelated to other types is suggested by the data on solubility.

The data reported here provide very strong evidence that the immobilization antigen, the antigen observed in gel diffusion, and the protein are all identical, and have indeed been purified. The experiments on proteolytic enzymes reinforce the conclusion that the antigens are proteins and show that the different antigens (D and A) may differ in their response to trypsin.

The different immobilization antigens are similar substances. They are all represented in the cell in about the same quantity, have about the same cellular distribution and diffusion coefficients, and are purified by the same procedures. They are similar in their unusually strong ability to induce antibody in the rabbit. Furthermore, their parallel cellular distributions and other properties suggest that their function, whatever it may be, is probably very similar in all serotypes. Nevertheless in some respects many of the immobilization antigens are very different—some show no serological cross reaction, they may have markedly different solubilities in ammonium sulfate and different sensitivities to trypsin. What these differences mean in terms of molecular structure, whether they reflect fundamental differences in amino acid sequence or in more gross patterns of cross-linkage and folding, is unknown. Since the differences in the antigens result from the expression of different genes, further elucidation of the nature of the differences between the antigens should yield information on the role of genes in determining protein structure.

SUMMARY

The immobilization antigens from different serotypes of *Paramecium aurelia* are closely related but distinct proteins. While many of the antigens are completely specific, others show cross reactions with other immobilization antigens. Such cross reactions are caused by the fact that although all of the antibody elicited by a given antigen can react with that antigen, some of it also can react with other serotype antigens. The pattern of cross reactions provides a means of

grouping related antigens. The immobilization antigens also differ in ammonium sulfate solubility. Furthermore, the pattern of solubility parallels the groupings based on serological specificity. The antigens differ in their sensitivity to trypsin.

ACKNOWLEGMENTS

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STUDIES ON THE PHENOMENON OF PREFERENTIAL SEGREGATION IN MAIZE[1]

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RHOADES (1942) demonstrated that in maize plants heterozygous for a type of chromosome 10 known as "abnormal 10", this chromosome undergoes preferential segregation during megasporogenesis. In 1945, LONGLEY reported that the phenomenon of preferential segregation is not confined to the abnormal chromosome 10, but that the chromosome induces preferential segregation of the other chromosomes of the complement if one homologue is knobbed and the other is knobless. However, there was little or no evidence to show whether the size of the knob is an important factor in preferential segregation. Therefore, one of the primary goals of this investigation was to determine the effect on preferential segregation of knobs of different sizes.

Experiments were also conducted to determine the effect of the abnormal chromosome 10 on preferential segregation and recombination in the long arm of chromosome 10, especially in the segment distal to the *r* locus.

MATERIALS AND METHODS

Some strains of maize have a chromosome 9 with a large terminal knob, others have knobs of smaller sizes, and some have a knobless chromosome 9. Knobs of three different sizes were selected for study (see FIGURES 1–3).

The largest knob (K^L) on chromosome 9 is approximately the size of the conspicuous heterochromatic piece found in the abnormal chromosome 10. The "medium"-sized knob (K^M) is one half to two thirds the length of the largest knob, while the smallest knob (K^S) involved in the experiments described below is approximately one fifth the size of the largest knob and about the same dimensions as that found on chromosome 9 in the inbred line, KYS. The knobless (k) chromosome 9 utilized in these experiments is, in reality, deficient for a very short segment of the end of the short arm. According to MCCLINTOCK (1944), the deficiency includes the terminal knob and part of the first adjacent chromomere.

The knobless chromosome described above is an ideal one for these experiments since it can be identified genetically as well as cytologically. When a plant is homozygous for this type of knobless chromosome 9, it is an albino *(wd wd)*, whereas an individual homozygous or heterozygous for the knobbed homologue *(Wd Wd* or *Wd wd)* and having nonmutant alleles in the *Wd* segment is always a green plant.

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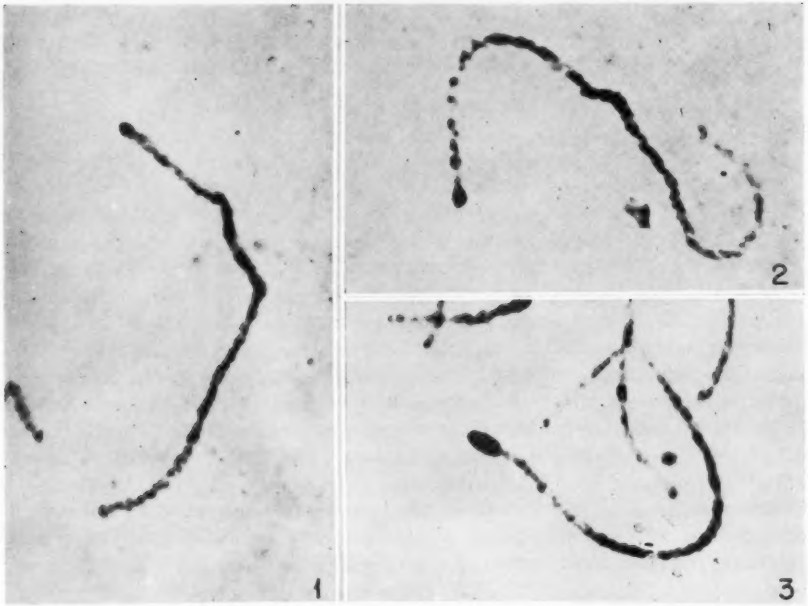

FIGURE 1.—Pachynema of a chromosome 9 bivalent homozygous for the small knob (K^S/K^S). FIGURE 2.—Pachynema of a chromosome 9 bivalent with one homologue having a "medium"-sized knob and the other knobless (K^M/k). FIGURE 3.—Pachynema showing a chromosome 9 bivalent homozygous for the large terminal knob (K^L/K^L).

The following gene symbols are used: A_1, colored aleurone, plant and pericarp; *c*, colorless aleurone; *r*, colorless aleurone and plant; g_1, "golden" plant; Lg_2, liguled leaf; *Py*, nonpigmy plant; sh_1, shrunken endosperm; sr_2, white striations on plant; *wd*, albino plant; *wx*, waxy endosperm; yg_2, yellow-green seedling and plant.

A variety of heterozygotes possessing knobs of different sizes in several combinations were tested. In all cases, the effect of varying knob size on the degree of preferential segregation is revealed by backcross ratios of genes linked to the knobs. In some of the compounds, the knobbed chromosome 9 is opposed by a knobless homologue while in other tests the homologues possess knobs of different sizes. The genes involved in these experiments include *wd*, yg_2, *c*, sh_1, and *wx* in various combinations as indicated. Of these, *wd* is the most distal and *wx* is closest to the centromere. In the study of preferential segregation and recombination in chromosome 10, the g_1, *r*, and sr_2 loci were employed. They are situated in the long arm of chromosome 10, sr_2 being the most distal locus. The abnormal chromosome 10 (K10) is derived from stocks used by RHOADES (1942, 1952) in his studies of preferential segregation.

TABLE 1

Results from backcrosses of K9/k9 compounds

Female parent	+ +	+ *wx*	*wd* +	*wd wx*	Total	Percent *Wd*	Percent *Wx*	Percent recomb.
a. K^S + +/k *wd wx* K10/k10	1372	669	419	992	3452	59.1**	51.9*	31.5
b. K^S + +/k *wd wx* k10/k10	2154	778	763	2035	5730	51.2	50.9	26.9
c. K^M + +/k *wd wx* K10/k10	3994	1543	757	2276	8570	64.6**	55.4**	26.8
d. K^M + +/k *wd wx* k10/k10	2598	582	518	2510	6208	51.2	50.2	17.7
e. K^L + +/k *wd wx* K10/k10	854	380	163	396	1793	68.8**	56.7**	30.3
f. K^L + +/k *wd wx* k10/k10	1555	213	225	1456	3449	51.3	51.6	12.7
g. K^L + *wx*/k *wd* + K10/k10	1442	3463	1726	581	7212	68.0**	56.1** (*wx*)	28.1

* Significant at the 5% level.
** Significant at the 1% level.

TABLE 2

Data obtained from backcrosses of K9/K9 compounds possessing knobs of different sizes

Female parent†	+ + +	*yg c sh wx*	+ *c sh wx*	*yg* + + +	+ + *sh wx*	*yg c* + +	+ + *wx*	*yg c sh* +	+ *c* + +	*yg* + *sh wx*	+ *c sh* +	*yg* + + *wx*	+ + *sh* +	*yg c* + *wx*	+ *c* + *wx*	*yg* + *sh* +	Total
a. K^L *sh wx*/K^M + +	1897	.....	.....	.....	2466	.....	435	.....	.....	....	.....	....	727	....	....	....	5525
b. K^M + + + +/K^S *yg c sh wx*																	
1. Pregermination	7030	.....	4763	.....	482	.....	1924	.....	208	....	886	....	20	....	10	....	15323
2. Postgermination	6199	2586	1100	624	366	180	1770	657	20	17	52	32	15	10	....	....	13628
c. K^M + + + +/K^S *yg c sh wx*	1655	704	409	173	128	68	488	192	3	2	18	19	10	4	....	....	3873
d. K^M + + + +/K^S *yg c sh wx*	512	487	114	91	22	29	114	116	2	2	8	5	2	0	....	....	1504
e. K^S *c sh wx*/k + + +‡	1157	.....	1828	.....	41	.....	344	.....	101	....	677	....	2	....	5	....	4155
f. K^M + + +/K^S *c sh wx*	2286	.....	1393	.....	134	.....	827	.....	79	....	423	....	4	....	5	....	5151

† Plants of entries a, b, c, e, and f are K10/k10 while those of entry d are k10/k10.
‡ Sib control cross for entry f.

RESULTS

Studies on preferential segregation in chromosome 9

The degree of preferential segregation of a small knob (K^S) against a knobless homologue was observed in plants heterozygous for abnormal chromosome 10 and in sib plants with only normal chromosome 10. As can be seen in the first two entries of Table 1, the *Wd* and *Wx* alleles linked with the knob were preferentially recovered in the presence of the abnormal chromosome 10 while random segregation for the two loci was observed in the sib control class homozygous for the normal chromosome 10. As previously reported by RHOADES (1942, 1952) using loci on chromosome 10, and by LONGLEY (1945) working with chromosome 9, loci further removed from the knob undergo a lesser degree of preferential segregation than those situated closer to the knob.

Recombination between the *Wd* and *Wx* loci was found to be 31.5 percent in the experimental class, while a value of 26.9 percent was observed in the sib control class. Although the difference between these two recombination values is small, it has been found to be statistically highly significant. The increase in the recombination frequency can be attributed to the presence of the abnormal chromosome 10. This statement finds support in the data of RHOADES and DEMPSEY (1957).

The third and fourth items in Table 1 (c and d) show the results of backcrosses of plants heterozygous for a knobless chromosome 9 and one carrying a medium-sized knob (K^M). The percentage values for the two loci studied deviate significantly from the expected 50 percent in plants with the abnormal chromosome 10, but normal backcross ratios occur in the class homozygous for the normal chromosome 10.

In the presence of the abnormal chromosome 10, a recombination value of 26.8 percent was realized. In the closely related control class, 17.7 percent recombination was found between *wd* and *wx*. This value is significantly lower than that found in plants heterozygous for the abnormal chromosome 10.

The fifth, sixth, and seventh entries in Table 1 involve compounds heterozygous for a large knob (K^L) on chromosome 9. In plants carrying abnormal chromosome 10, values of 68.8 percent *Wd* and 56.7 percent *Wx* were found when the genes were in coupling and values of 68.0 percent *Wd* and 56.1 percent *wx* when the markers were in repulsion phase. Therefore, it is the knob on chromosome 9, and not the alleles associated with the knob, that is responsible for the preferential segregation of chromosome 9 when the abnormal chromosome 10 is present in the megasporocyte. As expected, the sib control class gave random segregation values for these loci.

In the control class there was 12.7 percent recombination in the *wd-wx* region, while a value of 30.3 percent was found in the sib class heterozygous for the abnormal chromosome 10. A value of 28.1 percent recombination was realized in the repulsion experiment. These values again show the effect of the abnormal chromosome 10 in increasing the frequency of crossing over.

It is important to note that there are marked differences in the control recombination values presented in Table 1. The highest value (26.9 percent) occurred in the data involving the smallest knob and the lowest value (12.7 percent) was associated with the large knob, while an intermediate value (17.7 percent) was found in plants heterozygous for the K^M knob. Thus, in the knobbed-knobless heterozygotes, there is a negative correlation of crossing over and knob size. In a situation where one chromosome is knobbed and the homologue is knobless, the knob might interfere with the intimate pairing and thus cause difficulty in crossing over. The larger the knob, the greater might be the disturbance in synapsis. Working with an interstitial knob on chromosome 3, RHOADES and DEMPSEY (1957) have shown that recombination in the *Lg-A* region is reduced when the knob is heterozygous. Pairing in chromosome 3 bivalents heterozygous for the knob was not as intimate as in those bivalents without the knob. The disturbance in synapsis is not surprising since the knob is interstitial, but in the case of chromosome 9 pairs, where the knob is terminal, interference with pairing seems less likely to occur. No obvious departure from the normal synaptic pattern was observed by the writer in the knob heterozygotes. However, since the sporocytes were not examined specifically for pairing between the homologues of chromosome 9, it is not possible at this time to conclude whether the reduction in recombination is due to a disturbance in synapsis or to some other cause.

In the presence of the abnormal chromosome 10, recombination frequencies are increased and in some cases strikingly so. Apparently, the abnormal chromosome 10 counteracts the suppressive action of the knob on chromosome 9. In the compound where the small knob was opposed by a knobless chromosome 9, the frequency of recombination observed in the experimental class was increased by 17 percent from that observed in the sib control class. When the medium knob was employed, the recombination frequency in the experimental class was approximately 50 percent higher than in the closely related control class. The recombination value of 30.3 percent for the class heterozygous for the abnormal chromosome 10 and for the K^L 9 chromosome is nearly 150 percent greater than the value of 12.7 percent observed in the sib control class. Why the increase in crossing over caused by the abnormal chromosome 10 is greatest when the K^L chromosome is present is difficult to understand. A point of interest is that in all three classes of knob heterozygotes the abnormal chromosome 10 increased the frequency of crossing over from three different control values to a value of about 30 percent.

According to the hypothesis of preferential segregation advanced by RHOADES (1942, 1952), the frequency with which the knobbed chromosome becomes incorporated into the functional basal megaspore would depend on the frequency of preferential segregation following such crossovers. As previously mentioned, the frequency of crossing over in the three groups of knob heterozygotes was about 30 percent. Yet, the segregation values observed for the *Wd* and *Wx* loci are not identical for the three knob groups. The recovery of these dominant alleles was highest in the large knob group and lowest in the small knob group. It would

seem, then, that preferential segregation following such crossovers must occur more frequently with the larger knob and less frequently as the size of the knob decreases. A possible explanation is that the large knob has a greater neocentric activity than the smaller knobs and is thus a more efficient agent in reaching the functional basal megaspore. This point is difficult to demonstrate since chromosome 9 cannot be distinguished from the other chromosomes of the complement at anaphase II of meiosis. It should be pointed out that the comparisons made among the three groups of knob heterozygotes, i.e., K^{8}/k *vs.* K^{M}/k, etc., are not among full sibs, but the strains are closely related and it is believed that the comparisons are justified.

LONGLEY (1945) reported that the knobbed chromosome of a pair of chromosomes 9 is preferentially recovered over the knobless homologue even in the absence of the abnormal chromosome 10. He suggested that the abnormal chromosome 10 merely accentuates preferential segregation. The fact that random segregation was observed in the controls of the three knob heterozygotes clearly indicates that the heterozygous knobs on chromosomes 9 do not in themselves determine preferential segregation since this results only when the abnormal chromosome 10 is present. The data of RHOADES and DEMPSEY (1957) lend support to the above statement. There is no convincing evidence that preferential segregation occurs when the abnormal chromosome 10 is not present.

The studies described above deal only with heterozygous bivalents composed of one knobbed and one knobless homologue. To test further the hypothesis that knob size has an effect on preferential segregation, experiments were conducted in which both of the homologues of chromosome 9 had knobs which, however, differed in size. The results of these experiments are given in Tables 2 and 3.

When plants of the constitution $K^{L}9$ *wx*/$K^{M}9$ *Wx*, K10/k10 were used as female parents in backcrosses to k9 *wx*, k10 plants, the recessive allele *wx* was recovered more frequently than the dominant *Wx* allele. In a total of 7682 kernels, 4151 (54 percent) were found to have the *wx* type of endosperm starch. Since the *wx* locus is some distance from the knob and would therefore give a value for the recovery of the $K^{L}9$ chromosome which is less accurate than values obtained with loci closer to the knob, the following experiment was carried out.

The K^{L} chromosome was genetically marked with the sh_{1} as well as the *wx* alleles, while the K^{M} chromosome carried the dominant alleles of these two genes. On the linkage map (see RHOADES 1955) the locus of the sh_{1} gene is 29 while that of the *wx* gene is 59. Since the sh_{1} locus is much closer to the knob (position 0) than the proximal *wx* locus, it is a more efficient marker in determining preferential segregation. In the backcross, 57.8 percent of the kernels were "shrunken" (sh_{1}) and 52.5 percent of the kernels were "waxy" (*wx*) (Table 3-a). Chi-square tests show that the deviations from the expected 50 percent value are highly significant.

A comparison of segregation at the *wx* locus in K^{L}/K^{M} and K^{L}/k heterozygotes shows that the allele located on the K^{L} chromosome was recovered with a higher frequency from the K^{L}/k compounds than from K^{L}/K^{M} plants. A *t* test indicates

TABLE 3

Summary of results from backcrosses of K9/K9 compounds possessing knobs of different sizes

Female parent†	Total	Percentage Yg_2	C	Sh_1	Wx	yg-c	c-sh	Recombination percentage sh-wx	yg-sh	c-wx	yg-wx
a. K^L *sh wx*/K^M + +	5525	...	...	57.8** (*sh*)	52.5** (*wx*)	...	..	21.0	...	...	...
b. K^M + + + +/K^S *yg c sh wx*											
1. Pregermination	15323	...	61.7**	59.9**	53.1**	...	4.7	18.5	...	22.8	...
2. Postgermination	13628	65.0**	61.7**	59.9**	53.1**	13.5	4.5	18.6	17.5	22.7	34.5
c. K^M + + + +/K^S *yg c sh wx*	3873	70.0**	63.9**	62.2**	54.7**	16.1	5.6	18.9	21.4	23.7	37.7
d. K^M + + + +/K^S *yg c sh wx*	1504	51.5	49.7	50.1	50.5	14.9	3.8	16.3	18.0	19.8	32.3
e. K^S *c sh wx*/k + + +‡	4155	...	62.8** (*c*)	61.3** (*sh*)	53.4** (*wx*)	...	3.6	24.7	...	28.0	...
f. K^M + + +/K^S *c sh wx*	5151	...	63.1**	62.1**	54.2**	...	4.3	24.4	...	28.4	...

** Significant at the 1% level.
† Plants of entries a, b, c, e, and f are K10/k10 while those of entry d are k10/k10.
‡ Sib control cross for entry f.

TABLE 4

Results from backcrosses of g_1 + + *K10/+* r sr_2 *k10 and* g_1 + + *k10/+* r sr_2 *k10 compounds made in two different seasons*

Female parent	g_1 ++	+ r sr_2	g_1 r sr_2	+++	g_1 + sr_2	+ r +	++ sr_2	g_1 r +	Total	Percentage g_1	R	Sr_2
a. K10/k10†	564	196	33	87	3	5	0	0	888	67.6**	73.6**	73.9**
b. k10/k10†	268	263	59	62	183	169	9	5	1018	50.6	51.3	49.5
c. K10/k10‡	1907	870	159	323	6	17	1	1	3284	63.1**	68.1**	68.4**
d. k10/k10‡	632	589	122	105	344	404	8	9	2213	50.0	49.2	52.0

** Significant at the 1% level.
† Backcrosses made in Winter.
‡ Backcrosses made in Summer.

that this difference is significant at the one percent level. This finding could be interpreted to mean that K^L is incompletely dominant to K^M in producing preferential segregation or that competition exists between the two knobs. Although the K^L/k and K^L/K^M plants were not sibs and therefore not strictly comparable, they possess many genes in common since both were derived from crosses involving the same K^L/K^L stock.

The only data at all comparable to those described above come from LONGLEY's (1945) work on chromosome 6. LONGLEY studied preferential segregation of a pair of chromosomes differing morphologically in that one possessed three knobs and the other only two. In addition, there was a size difference in one of the knobs common to both chromosomes. The larger knob was located on the homologue with the three knobs as was the dominant *Py* allele. In this study the *Py* allele was recovered with a frequency of 67.2 percent. Since both homologues differ in two respects, it is very difficult to determine whether the knob-knobless condition or the large knob-small knob condition (or possibly both) is responsible for the preferential recovery of the *Py* allele. Furthermore, the location of the *Py* locus in relation to the knobs is not known.

The behavior of combinations of knobs in preferential segregation has been more precisely studied in tests involving a marker situated close to the knob as well as three more distant loci. The chromosome 9 with the larger of the two knobs (K^M) carried the dominant alleles Yg_2, *C*, Sh_1, and *Wx*. According to the best available linkage map (RHOADES 1955), the yg_2 locus is located seven map units from the terminal knob, while *c*, sh_1, and *wx* are located 26, 29, and 59 map units from the knob, respectively. The chromosome with the smaller knob (K^S) carried the recessive alleles of the genes listed above.

In the backcrosses involving the abnormal chromosome 10 (Tables 2 and 3-b, c, and f) deviations from a 1:1 ratio were found for all four genes, with the genes on the K^M chromosome being recovered more frequently. The genes nearest the knob showed the highest frequencies of preferential segregation. Normal backcross ratios occurred in the backcrosses of plants with normal chromosome 10 (Tables 2 and 3-d).

In Table 3-c the percentage of plants of the Yg_2 phenotype was 70.0 percent, a value similar to that found for the *Wd* phenotype in plants of K^L9 *Wd*/k9 *wd*, K10/k10 constitution (Table 1). The degree of preferential segregation as followed by these pseudoallelic genes is expected to be greater in the K^L/k compounds than in the K^M/K^S plants because the difference between the two homologues in knob size is greater in the former than in the latter compound. The unexpected similarity in these values may be due to environmental differences since the data of the K^L/k compounds came from backcrosses made in the summer of 1957 while the K^M/K^S plants of Table 3 were grown in the winter of 1958. The value for preferential segregation of the Yg_2 locus found in the other K^M/K^S family (Table 3-b) was somewhat less than that given in Table 3-c. The backcrosses of entries b, e, and f of Table 3 were made during the summer of 1957.

The segregation values observed for the K^M/K^S compounds (Table 3-b) are

comparable to those of the K^M/k compounds (Table 1). The K^S and k chromosomes are recovered from these compounds with approximately equal frequencies, although in K^S/k plants the K^S chromosome is favored. It would seem that the larger knob is dominant to the smaller one in K^M/K^S heterozygotes.

More critical evidence on interaction of knobs of different sizes comes from the data in Table 3-e and f where sib comparisons of K^M/K^S and K^S/k heterozygotes are available. Here the frequencies with which the alleles in the larger knobbed chromosomes were recovered are essentially the same. Since it has been found that larger knobs give higher values of preferential segregation than do smaller ones, it would be expected that values from the K^M/K^S compounds would be greater than those from K^S/k heterozygotes if K^M were completely dominant. Such, however, was not the case and the data suggest that K^M is incompletely dominant to K^S.

The recombination frequencies presented in entries c and d of Table 3 indicate that plants carrying the abnormal chromosome 10 undergo a somewhat higher degree of crossing over than those with normal chromosomes 10. The following recombination values were obtained in the control (k10/k10) class and in sibs heterozygous for abnormal chromosome 10:

	k10/k10	K10/k10	Difference
yg–c:	14.9%	16.1%	1.2%
c–sh:	3.8	5.6	1.8
sh–wx:	16.3	18.9	2.6
yg–sh:	18.0	21.4	3.4
c–wx:	19.8	23.7	3.9
yg–wx:	32.3	37.7	5.4

A total of 40.6 percent recombination (sum of the frequencies in the *yg-c*, *c-sh*, and *sh-wx* regions) was observed in the experimental class and a value of 35.0 percent recombination was observed in the sib control class. This is in agreement with the data previously cited.

Studies on recombination and preferential segregation in chromosome 10 in the presence of the abnormal knob

In 1942, RHOADES reported that approximately one percent crossing over occurs between the *r* locus and the extra piece which identifies the abnormal chromosome 10. E. G. ANDERSON (see RHOADES 1952) had found between four and five percent recombination between *r* and a distally placed break in the long arm of the normal chromosome 10 in a translocation heterozygote. Both ANDERSON and RHOADES pointed out the possibility of the existence of a considerable amount of crossing over in the segment distal to the *r* locus in normal chromosome 10. JOACHIM and BURNHAM (1955) found that the "striate" (or Waseca Stripe) gene was 21.4 to 31.0 recombination units distal to the *r* locus. JOACHIM (1956) confirmed this earlier finding and found recombination ranging from 25.1 to 31.5 percent be-

tween *r* and sr_2. She also verified their earlier finding that the order of g_1, *r*, and sr_2 on the long arm of chromosome 10 is as here reported.

In the present experiment, the abnormal chromosome 10 was marked with the recessive allele g_1 and the dominant alleles *R* and Sr_2, and the normal chromosome 10 was marked with the dominant allele G_1 and the recessive alleles *r* and sr_2. Female parents of such a constitution and controls possessing two normal chromosomes 10 were backcrossed to male parents homozygous for the three recessive alleles. The results obtained in the summer and winter seasons of 1957 are given separately in Table 4. The plants used as females in both instances were from kernels taken from the same ear. Comparisons of K10/k10 with k10/k10 plants in both sets of data show significant deviations from 50 percent for the three loci in the K10/k10 classes only. The highest values are obtained for the Sr_2 locus, which is closest to the knob.

The following recombination values were found in the class heterozygous for the abnormal chromosome 10 and in the sib control class:

	Winter		Summer	
	K10/k10	k10/k10	K10/k10	k10/k10
g–r:	13.5%	13.3%	14.7%	11.0%
r–sr:	0.9	36.0	0.8	34.6
g–sr:	14.4	46.5	15.4	44.1
Double crossovers:	0.0	1.4	0.1	0.8

As shown above, there is a drastic reduction in the recombination frequency when the abnormal chromosome 10 is present in the heterozygous condition. In its absence a considerable amount of recombination occurs in the *r*-sr_2 region. The reduction observed in the experimental class is not surprising since there is non-homology in chromosome structure between the abnormal chromosome 10 and the normal one in the distal one sixth segment of the long arm of normal chromosome 10. In spite of the non-homology and absence of crossing over in this segment, pairing, as determined cytologically, is intimate. Although there is a slightly higher frequency of crossing over in the *g*-*r* region in plants with the abnormal chromosome 10 than in those with the normal chromosome, the difference is not statistically significant.

The frequencies with which the three genes were recovered in the summer experiment (Table 4-c) are somewhat lower than those found in the winter (Table 4-a) as shown by the following comparison:

	K10/k10	
	Summer	Winter
g_1	67.6%	63.1%
R	73.3	68.1
Sr_2	73.9	68.4

Since the F_1 kernels used in the two sets of experiments were from the same ear, the differences cannot be attributed to genetic variations but must be due to

environmental differences in the two growing seasons. The recombination frequencies obtained in the two seasons, however, are quite similar as shown below:

	K10/k10	
	Summer	Winter
g–r	13.5%	14.7%
r–sr	0.9	0.8
g–sr	14.4	15.4
Double crossovers	0.0	0.1

The only obvious difference between the two experimental classes is the absence of double crossovers in the summer group. The recombination values in the sets of data are not significantly different, but the frequency of preferential segregation is higher in one than in the other. If the hypothesis is accepted that preferential segregation occurs only when appropriate crossovers produce heteromorphic dyads, it would seem that similar crossover frequencies should result in like frequencies of preferential segregation. Since this was not the case, it follows that preferential segregation does not invariably occur when heteromorphic dyads are present. It would not be surprising to find variation in the behavior of heteromorphic dyads, possibly dependent on the amount of neocentric activity, associated with changes in the growing season.

Of the 25 crossovers in the *r-sr* region (Table 4-c), 17 were of G_1-*r*-Sr_2 constitution and one was of the g_1-*r*-Sr_2 constitution. These crossover plants were analyzed cytologically to determine where the exchange occurred. All were heterozygous for the abnormal chromosome 10 and possessed the three small chromomeres which are found in the proximal section of the foreign piece. Thus, crossovers between *r* and sr_2 occur to the left of the three chromomeres (see Figure 4). This is in agreement with RHOADES' observation (1942) that crossovers between the *R* locus and the foreign segment occur in a region proximal to the three chromomeres. Crossing over, apparently, is restricted to regions which are truly homologous.

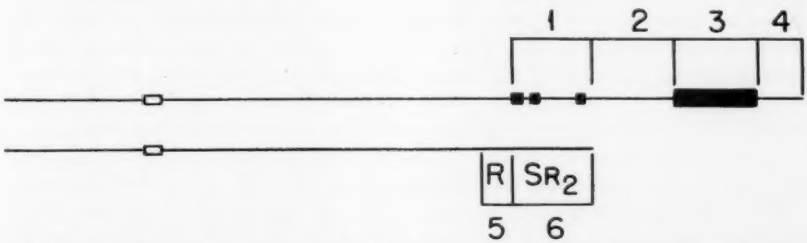

FIGURE 4.—Diagram of a chromosome 10 bivalent heterozygous for the abnormal chromosome 10 showing approximate locations of *R* and Sr_2. 1 = distal one sixth segment in which crossing over is inhibited, 2 = euchromatic segment, 3 = heterochromatic segment, 4 = distal euchromatic segment, 5 = region in which *r*-sr_2 crossovers occur, and 6 = region containing the Sr_2 locus.

It has been shown that exchanges in the *r-sr* region are drastically reduced in K10/k10 heterozygotes and that they probably do not occur in the distal one sixth segment of 10L. These observations have some bearing on the cytological position of the Sr_2 locus. Two possibilities exist: (1) either Sr_2 is found to the left of the distal one sixth segment or (2) it is located within this region. The available evidence favors the latter position although no certain conclusion can be reached at present. Studies with deficiencies for the *R* locus (STADLER 1933) have shown that *R* is placed very close to the distal one sixth segment. Since, in normal chromosome 10 bivalents, Sr_2 lies at least 35 units beyond *R*, it is logical to assume that it is located within the distal one sixth segment. Moreover, the restriction of crossing over between *R* and Sr_2 to the segment to the left of the three chromomeres readily accounts for the low percentage of recombination for this region in K10/k10 plants. It is likely that the structural dissimilarity existing between the distal one sixth segment of normal chromosome 10 and the corresponding segment of abnormal chromosome 10 is responsible for the great reduction in recombination in the *r-sr* region. If the morphologically different segment of the abnormal chromosome 10 did not contain loci normally present in chromosome 10, stocks homozygous for abnormal chromosome 10 would be deficient for the loci in the distal segment. Deficiencies in this segment are not tolerated in the male gametophyte as has been shown by translocation studies (ANDERSON, unpublished data).

GENERAL DISCUSSION

There is little doubt that considerable variation exists in the processes leading to preferential segregation in different organisms. In *Drosophila melanogaster*, for example, NOVITSKI (1951), NOVITSKI and SANDLER (1956), and ZIMMERING (1955), working with heteromorphic bivalents, have shown that the chromosome recovered with the higher frequency is always the shorter of the two homologues. In maize the reverse is true. Yet, the formation of heteromorphic dyads as a consequence of crossing over is a necessary prerequisite to preferential segregation in both species. Neocentromeres, which are held responsible for preferential segregation in maize, have not been reported in Drosophila. According to NOVITSKI (personal communication), the non random orientation of the chromatids, which persists through the meiotic divisions, is established at anaphase I. It is believed that the shorter chromatids of heteromorphic dyads are directed toward the poles because they offer less mechanical resistance to the poleward movement.

Another indication that preferential segregation in maize and Drosophila may be determined by divergent mechanisms comes from the study of ZIMMERING (1955). He found that complementary crossover chromatids in certain translocation heterozygotes are not recovered from females with equal frequencies while the noncrossover chromatids occur in the expected ratio of 1:1. The data from maize, on the other hand, show deviations from a 1:1 ratio in all classes, crossover and noncrossover (Table 5). It is not surprising to find preferential segregation among the noncrossover chromatids even though only bivalents with

TABLE 5

Comparisons of the ratios obtained in the noncrossover and crossover classes for all knob combinations studied. In each instance, the value shown is the frequency of the reciprocal class containing the genetic marker closest to the "preferred" knob

Combination			Class involving a crossover in region							
			Non	*yg-c*	*c-sh*	*sh-wx*	*yg-c+c-sh*	*yg-c+sh-wx*	*c-sh+sh-wx*	*yg-wx*	*wd-wx*
a. K^L/k	*K*10/k10	(Table 1-g)	0.67	...	...	...	...	...	...	...	0.71
b. K^L/k	*K*10/k10	(Table 1-e)	0.68	...	...	...	...	...	...	...	0.70
c. K^L/k	k10/k10	(Table 1-f)	0.51	...	...	...	...	...	...	...	0.49
d. K^L/K^M	*K*10/k10	(Table 2-a)	0.57	...	...	0.63	...	...	...	...	...
e. K^M/k	*K*10/k10	(Table 1-c)	0.64	...	...	...	...	...	...	...	0.67
f. K^M/k	k10/k10	(Table 1-d)	0.51	...	...	...	...	...	...	...	0.53
g. K^M/K^S	*K*10/k10	(Table 2-f)	0.62	...	0.63	0.66	...	...	0.56	...	...
h. K^M/K^S	*K*10/k10	(Table 2-c)	0.70	0.70	0.65	0.72	0.60	0.49	0.71	0.70	...
i. K^M/K^S	k10/k10	(Table 2-d)	0.51	0.56	0.43	0.50	0.50	0.62	1.00	0.51	...
j. K^M/K^S	*K*10/k10	(Table 2-b)	0.71	0.64	0.67	0.73	0.54	0.62	0.60	0.70	...
k. K^S/k	*K*10/k10	(Table 2-e)	0.61	...	0.71	0.66	...	...	0.71	...	...
l. K^S/k	*K*10/k10	(Table 1-a)	0.58	...	...	...	...	...	...	...	0.62
m. K^S/k	k10/k10	(Table 1-b)	0.51	...	...	...	...	...	...	...	0.51
n.	*K*10/k10	(Table 4-a)	0.74	0.73 (*g-r*)		0.62 (*r-sr*)	... (*g-r* + *r-sr*)		...	...	...
o.	k10/k10	(Table 4-b)	0.50	0.53		0.49	0.64		...	...	...
p.	*K*10/k10	(Table 4-c)	0.61	0.67		0.74	0.50		...	...	...
q.	k10/k10	(Table 4-d)	0.52	0.46		0.54	0.47		...	...	...

a crossover between the knob and the centromere undergo this phenomenon. Knobbed crossover and knobbed noncrossover chromatids are recovered with equal frequencies and in excess of the knobless strands from bivalents with chiasmata. If the proportion of such bivalents is relatively high, the 1:1 ratio of K:k strands from noncrossover bivalents would be altered by the large contribution of knobbed noncrossover strands from bivalents with chiasmata. In Drosophila translocation studies the appropriate chiasma occurs with a frequency of 12–15 percent and therefore has little influence on the ratio of long to short chromatids in the noncrossover class.

In the chromosome 9 studies of maize it is possible to estimate the expected departure from a 1:1 ratio within each crossover and noncrossover class. If it is assumed that preferential segregation of the knobbed chromatid occurs in 70 percent of the heteromorphic dyads and if reasonable chiasma frequencies are adopted (e.g., Table 5-k: 14 percent, one chiasma in the *wx* centromere region; 49 percent, one chiasma between *sh* and *wx;* 7 percent, one chiasma in the *c-sh* region; 31 percent, no chiasma), values are obtained which agree well with those shown in Table 5.

The effect of heterochromatin on segregation in Drosophila has been studied by STURTEVANT (1936) using the essentially heterochromatic chromosome IV. It was possible to identify 26 kinds of chromosome IV by testing individual chromosomes from different sources in triplo-IV females. The chromosomes IV were found to differ in their propensity to segregate with a specific member of the trivalent. In maize, differences in preferential segregation are probably associated with quantitative rather than qualitative differences in heterochromatin though the latter possibility cannot be disproven. Although knobs are sites of neocentric activity, RHOADES (1952) has shown that they do not form neocentromeres unless a true centromere is present on the chromosome. He believes that a substance from the true centromere accumulates near the knob, which either behaves as a physical barrier or actively attracts and absorbs this material. It is conceivable that a larger knob may be more effective as a block to the "flow" of the centric substance and therefore neocentric activity is increased. It is obvious that knobs play a vital role in preferential segregation, but the means by which they respond to the introduction of the abnormal chromosome 10 are not clear.

It has been demonstrated by RHOADES and confirmed by the writer that crossing over is increased in the presence of the abnormal chromosome 10. Apparently this effect is independent of preferential segregation and the production of neocentromeres since recombination is increased in bivalents which possess no knobs (RHOADES and DEMPSEY 1957) and therefore are not undergoing preferential segregation. REES (1955) has shown the existence of genes in the rye genome which influence the rate of crossing over as determined by the frequency of chiasma formation. It is possible that genetic factors controlling crossing over are situated in the non-homologous distal segment of the abnormal chromosome 10. In addition to the increased recombination induced by the abnormal chromosome 10, the data on chromosome 9 indicate that the extent of increase is greatly

influenced by the size of the knob on chromosome 9. Following introduction of the abnormal chromosome 10, the *wd-wx* recombination was altered from 12.7 percent to 30.3 percent in K^L/k plants, from 17.7 percent to 26.8 percent in K^M/k plants, and from 26.9 percent to 31.5 percent in K^S/k compounds. It is difficult to understand why there should be such a great variation in the extent to which crossing over is increased, unless the 30 percent recombination achieved in each case represents a maximum level which cannot be exceeded.

The frequency with which the knobbed homologue in a heteromorphic bivalent reaches the basal megaspore in plants carrying the abnormal chromosome 10 depends on two variables: (1) the frequency of crossing over between the knob and the centromere and (2) the frequency of preferential segregation following such a crossover. It has been shown that the percent of crossing over in chromosome 9 is increased in the presence of the abnormal chromosome 10 and that the amount of the increase is influenced by the size of the knob (K9). The frequency of preferential segregation is also variable, as can be seen in the experiments where recombination is constant. For example, the recovery of the K9 chromosome is increased in K^L/k compounds over K^S/k compounds in the presence of the abnormal chromosome 10. Since the *wd-wx* recombination is about 30 percent in both cases, it seems probable that there is a higher degree of preferential segregation in the first class of plants. A second example where different degrees of preferential segregation can be observed comes from the chromosome 10 data. Different frequencies of recovery of the *R* and Sr_2 alleles were obtained from plants grown in the field and in the greenhouse. Since the recombination values were the same for the two experiments, it has been concluded that differences exist in the behavior of the chromosomes following such crossing over, perhaps, related to the extent of neocentric activity. Thus, preferential segregation can be influenced by amounts of heterochromatin present on the bivalent in question and probably also by environmental factors associated with different growing seasons.

SUMMARY

Since the investigations of RHOADES and LONGLEY with abnormal chromosome 10 in maize have demonstrated the active participation of knobs in the phenomenon of preferential segregation, it was deemed of interest to ascertain the effect of knob size on the degree of preferential segregation. For such a study, three stocks differing in the size of the terminal knob on the short arm of chromosome 9 were utilized. Compounds were derived in which the knobbed chromosome 9 was opposed by a knobless homologue, while in other plants the homologues possessed knobs of different sizes. In all cases, the effect of varying knob size was determined by backcross ratios of genes linked to the knobs. Experiments were also conducted to determine the extent of preferential segregation and recombination among loci situated in the long arm of chromosome 10, specifically in the segment distal to the *r* locus. In the above experiments, the abnormal chromosome

10 utilized was derived from stocks used by RHOADES in his experiments on preferential segregation.

The results of the investigation reveal the following points:

1. The degree of preferential segregation of a knobbed chromosome 9 over a knobless one becomes progressively greater with increase in knob size.

2. The incidence of crossing over in chromosome 9 is increased in the presence of the abnormal chromosome 10 and the amount of increase is influenced by the size of the knob on chromosome 9. The data are in agreement with the findings of RHOADES and DEMPSEY that crossing over is increased in the presence of the abnormal chromosome 10.

3. In the three kinds of knobbed-knobless heterozygotes, the abnormal chromosome 10 increases the frequency of crossing over in the *wd-wx* region to approximately the same level (30 percent) irrespective of knob size.

4. In the knobbed-knobless heterozygotes homozygous for normal chromosome 10, there is a negative correlation between crossing over and knob size.

5. When members of a pair of chromosomes 9 have knobs of different sizes, the chromosome with the larger knob is recovered more frequently. There is evidence of competition or incomplete dominance between knobs of different sizes.

6. Evidence supports the contention that preferential segregation can occur only in the presence of the abnormal chromosome 10.

7. Crossing over between *r* and the most distal marker, sr_2, in the long arm of chromosome 10 is drastically reduced in bivalents heterozygous for the abnormal chromosome 10. Crossovers between these two genes in plants heterozygous for the abnormal chromosome 10 always occur to the left of the three chromomeres of the abnormal chromosome 10, indicating that no crossing over takes place in the non-homologous regions of the two homologues.

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EARLY GENERATION ANALYSIS OF LENGTHS OF HETEROZYGOUS CHROMOSOME SEGMENTS AROUND A LOCUS HELD HETEROZYGOUS WITH BACKCROSSING OR SELFING

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WHEN a locus is held heterozygous for n generations of backcrossing, the average length of heterozygous segments surrounding the selected locus asymptotically approaches $2/n$ (FISHER 1949). With the equivalence of eight to ten generations of backcrossing this criterion is adequate. Experiments which involve early generation tests with genetically similar or "isogenic lines", chromosome substitution studies, factor transfer or the development of genetic stocks require a more exact criterion for heterozygous segment lengths for the early generations of inbreeding. The objective of this paper is to develop a distribution of the segment lengths heterozygous about a locus held heterozygous for any number of generations of backcrossing or selfing.

Derivation of expected length and variance of lengths heterozygous after selected generations of inbreeding

Consider a chromosome of length s measured with reference to the true genetic map scale (FISHER 1948) and the conditions (i) that crossovers occur independently and (ii) that the locus Aa which is being held heterozygous is in the center of the chromosome. The restriction (ii) is not essential but simplifies appreciably the derivation. The resulting calculations will serve as an adequate guide for the interpretation of the segment analysis. Let c be any length measured with reference to the true genetic map scale ($0 \leqslant c \leqslant s$). Then the transformation, $t = c/s$, reduces the measurement of a segment to a proportion of the chromosome. The length of any chromosome is 1.0 and the locus of Aa is 0.5, with reference to the proportionate scale. The length of segment t will be measured only from the left of the Aa locus. Thus, $0 \leqslant t \leqslant \frac{1}{2}$, since only the proportion of a segment occurring in this half of the chromosome will be considered. For further discussion, these lengths will be defined as half lengths. Our first problem will be to determine the cumulative distribution for t, $F(t)$, in the chromosomes arising from a meiotic division, considering only half lengths as defined.

The true genetic map scale is the expected number of breaks per unit length. Chromosomes resulting from a meiotic division may contain 0,1, . . . ,x, . . . breaks or points of genetic recombination. The distribution of x is defined by some discrete distribution. With the assumption of independence the probability P_x, that a chromosome will contain x breaks may be defined by the Poisson dis-

tribution, $P_x = (e^{-s}s^x/x!)$, where $s = E[x]$ is the length of the chromosome (HANSON 1959). The probability density for the x breaks within a chromosome will be uniform.

The derivation of $F(t)$ can best be illustrated by considering specific cases. Consider as a set, A_3, all possible chromosomes which involve three breaks. The probability that any chromsome will occur in this set can be shown to be P_3. Each chromosome of this set will contain four segments identified by sequence, 0 to γ_1, γ_1 to γ_2, γ_2 to γ_3, and γ_3 to 1.0, where γ_i represents the locus of a break. These segments are identified by subsets, A_{31}, A_{32}, A_{33}, and A_{34}, respectively. A segment in subset A_{31} will contain the *Aa* locus only if $\frac{1}{2} < \gamma_1 < \gamma_2 < \gamma_3 < 1.0$, which constitutes $\frac{1}{8}$ of the elements in the subset A_{31}. However, the length of t is $\frac{1}{2}$ for all elements of this restricted subset since only half-lengths are considered. A segment in subset A_{32} will contain the *Aa* locus only if $0 < \gamma_1 < \frac{1}{2}$, $\frac{1}{2} < \gamma_2 < \gamma_3 < 1.0$ which constitutes $\frac{3}{8}$ of the elements of subset A_{32}. The probability that a randomly selected half length in this restricted sample will be less than or equal to t can be shown to be $1-2(\frac{1}{2}-t)$. Similarly, it can be shown that $\frac{3}{8}$ of the elements in subset A_{33} will contain the *Aa* locus with a corresponding probability, $1-2^2(\frac{1}{2}-t)^2$. The corresponding values for A_{34} are $\frac{1}{8}$ and $1-2^3(\frac{1}{2}-t)^3$.

Considering all possible subsets, one can construct the following recurrent relationship for the cumulative distribution of half lengths (t):

$$F(t) = \sum_{x=1}^{\infty} \sum_{r=2}^{x+1} (\tfrac{1}{2})^x P_x \binom{x}{r-1} [1-2^{r-1}(\tfrac{1}{2}-t)^{r-1}], \quad 0 \leqslant t < \tfrac{1}{2},$$

$$Pr(t = \tfrac{1}{2}) = \sum_{x=0}^{\infty} (\tfrac{1}{2})^x P_x.$$

The distributions can be simplified as follows:

$$F(t) = 1 - \sum_{x=0}^{\infty} P_x(\tfrac{1}{2})^x(1+2Z)^x \qquad , 0 \leqslant t < \tfrac{1}{2}$$

$$Pr(t = \tfrac{1}{2}) = \sum_{x=0}^{\infty} P_x(\tfrac{1}{2})^x,$$

where $Z = (\frac{1}{2}-t)$. With the assumption of independence, P_x can be defined by the Poisson distribution, and the following simplified expressions will result:

$$F(t) = 1 - e^{-s(\frac{1}{2}-Z)} \qquad , 0 \leqslant t < \tfrac{1}{2},$$
$$Pr(t = \tfrac{1}{2}) = e^{-s/2}.$$

$F(t)$ is discontinuous at $t = \frac{1}{2}$. $F(t)$ represents the cumulative distribution of half lengths, as measured from the locus of *Aa* (0.5), in the chromatids generated by a meiotic division. The probability that a crossover will not occur within a region from the *Aa* locus is $1-F(t)$, t being the length of the segment.

The procedure for determining the cumulative distribution, $G(t_n)$, for half lengths which are heterozygous after n generations of backcrossing follows the procedure given by FISHER (1949, p. 50),

$$G(t_n) = 1 - (1 - F(t))^n \qquad , 0 \leqslant t < \tfrac{1}{2},$$

which reduces to

$$G(t_n) = 1 - e^{-snt} \qquad , 0 \leqslant t < \tfrac{1}{2}.$$

The expected value of t^m after n generations of backcrossing is:

$$E[t^m] = \int_0^{\frac{1}{2}} t^m dG(t_n) + (\tfrac{1}{2})^m (e^{-ns/2}),$$

noting that $F(t)$ is discontinuous at $t = 1/2$. Since the exact cumulative distribution of $F(t)$ is known and the limits of integration are defined, the segment lengths can be evaluated for all generations of backcrossing. Since t represents a proportion of a chromosome, the expectations can be expressed in terms of the genetic map units by the conversion $s^m E[t^m]$.

It should be noted that for selfing, $n = 2n' - 2$, where n' represents the filial generation, since crossing over in either male or female gametes will effectively reduce the length of heterozygous segment around the *Aa* locus. The expected half lengths together with the variance of half lengths for chromosome of lengths 1/2, 1.0, and 2.0 are given in Table 1. As n becomes large, the expected length and variance expressed in genetic map units for half lengths which are heterozygous approach $1/n$ and $1/n^2$, respectively. The entries in Table 1 must be doubled to include the lengths on both sides of the *Aa* locus. The initial increase in $E[V(c)]$ is attributed to the high proportion of the long lengths, $t = 1/2$, in the original chromatid array.

TABLE 1

The expected half length (centimorgans/100) and variance of heterozygous chromosome segments associated with a locus held heterozygous in backcrossing or selfing for selected chromosome lengths and generations of inbreeding

Length chromosome	Stat.*	Generation									
		BC_1	BC_2 F_2	BC_3	BC_4 F_3	BC_5	BC_6 F_4	BC_8 F_5	BC_{10} F_6	BC_{12} F_7	BC_{20} F_{11}
.5	$E[c]$	.221	.197	.176	.158	.143	.129	.108	.092	.079	.050
	$E[V(c)]$	.0041	.0064	.0076	.0081	.0081	.0078	.0068	.0058	.0048	.0023
1.0	$E[c]$	.393	.316	.259	.216	.184	.158	.123	.099	.083	.050
	$E[V(c)]$	.0256	.0322	.0312	.0275	.0233	.0194	.0133	.0093	.0067	.0025
2.0	$E[c]$	.632	.432	.317	.245	.199	.166	.125	.100	.083	.050
	$E[V(c)]$	.1289	.1101	.0776	.0533	.0373	.0270	.0155	.0100	.0069	.0025
Limiting Function	$E[c]$	1.000	.500	.333	.250	.200	.167	.125	.100	.083	.050
	$E[V(c)]$	1.0000	.2500	.1111	.0625	.0400	.0278	.0156	.0100	.0069	.0025

* $E[c] = sE[t] = \frac{1}{n}[1 - e^{-sn/2}]$ (centimorgans/100).

$E[c^2] = s^2E[t^2] = \frac{1}{n^2}[2 - (sn+2)e^{-sn/2}]$ (centimorgans/100)^2.

DISCUSSION

The exact distribution for the heterozygous half lengths measured from a gene pair held heterozygous during a backcrossing or selfing program has been presented. Half lengths have been defined as lengths measured in only one direction from the fixed locus. The locus of the factor pair was selected as the center of the genetic map, and the derivations were made on the assumption of no interference. The results are applicable to the early generations of backcrossing or selfing but are limited by the two restrictions stated. As the number of generations of backcrossing or selfing becomes large, the limitations imposed by the two restrictions are inconsequential. The statistics given in Table 1 must be doubled to define the distribution of lengths surrounding the fixed locus which is still heterozygous after selected levels of inbreeding.

The relationships developed by FISHER (1949) and by BARTLETT and HALDANE (1935) are limiting functions. The limiting functions for the expected half lengths $(1/n)$ and the variance of half lengths $(1/n^2)$ have been included in Table 1 for comparative purposes. As might have been expected, the shorter the genetic map length for a chromosome the greater will be the error in using the limiting values to estimate the mean and variance of half lengths in the early generations of inbreeding. For a chromosome of map length 1.0, which could represent an average genetic map length of a chromosome, the limiting values appear adequate after the equivalence of about eight generations of backcrossing.

The statistics given in Table 1 represent the expected mean and variance of the half lengths occurring in independent lines where the locus has been held heterozygous through selected generations of backcrossing or inbreeding. It is evident that the heterozygous chromosome segments after eight to ten generations of backcrossing are of considerable length. This information can be used in the designing of experiments to study the association of quantitative or qualitative characters with the fixed locus (Aa). Further, since in any one line considerable sampling variation may occur in heterozygous segment lengths associated with the Aa locus, the variances given in Table 1 can be used to determine the number of independent lines which must be carried to the selected level of inbreeding so that a desired reproducibility is obtained in the segment surrounding the fixed locus. Table 1 can thus be used as a guide to determine the adequacy of sampling in the early generation testing of "isogenic lines".

SUMMARY

The cumulative distribution for the heterozygous half lengths from a gene pair held heterozygous during a backcrossing or a selfing program was developed. Since the development involved the cumulative distribution for heterozygous half lengths following a meiotic division, the average heterozygous segment length and the expected variance for heterozygous segment lengths were available for the early generations of backcrossing or selfing. The statistics were evaluated for selected chromosome map lengths, and the restrictions required for

the development were discussed. The information presented would be pertinent for the designing of experiments involving early generation testing of genetically similar or "isogenic lines".

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THEORETICAL DISTRIBUTION OF THE INITIAL LINKAGE BLOCK LENGTHS INTACT IN THE GAMETES OF A POPULATION INTERMATED FOR n GENERATIONS

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IN the initial phases of breeding for the improvement of yield and quality in a crop species, progress normally was quite rapid. However, as the breeding program progresses, additional genetic gains over existing genotypes become difficult to achieve. An accumulation of desirable germplasm in the existing superior lines would adequately explain the plateau in genetic progress but would also represent a limit for genetic progress. As a result of the freedom of exchange of genetic source material between workers, the superior lines for a species frequently represent a set of genotypes with quite similar parental backgrounds. Breeding programs involving these superior lines result primarily in the reshuffling of a restricted pool of genetic factors.

A current example is the breeding program for the improvement of soybean varieties. An intensified breeding program in soybeans began in the early 1930's. Initial progress was rapid; however, at present genetic gains with each new cycle of breeding are becoming more difficult to achieve. The select varieties which represent the elite genotypes also have many common parentages. A re-evaluation of present breeding procedures is required to facilitate a more rapid genetic progress beyond the present plateau (JOHNSON 1954).

The extent to which linkage blocks can be broken if a gene pool were held in a heterozygous state for a number of generations is information which would be extremely helpful in evaluating breeding procedures, especially for a self-pollinated species in which intercrossing could be costly. The objective of this paper is to develop and to interpret the theoretical distribution of the lengths of initial linkage blocks still intact in the gametes of a population intermated for n generations.

Distribution of lengths of linkage blocks

Segment lengths are measured with respect to the true genetic map scale. The true genetic map distance for a segment length is the expected number of breaks or points of genetic recombination for the segment (FISHER 1948). A genetic map length for a chromosome will be taken as s. The length of any segment will be defined as c, $0 \leqslant c \leqslant s$. The transformation $t = c/s$ places all measurements on a proportionate scale. Thus, a chromosome length is 1.0 and $0 \leqslant t \leqslant 1.0$. In the following derivations segment lengths will be measured with reference to the proportionate scale.

Consider a population synthesized by intermating a selected group of individuals. For this paper the population in any generation will be considered to be of sufficient size so that the probability of a genetic recombination involving identical linkage blocks from one of the original progenitor chromosomes is small and can be ignored. The chromosomes in the gametes which give rise to the first generation will be taken as the progenitor chromosomes with corresponding initial linkage blocks. Thus, the reduction in the average linkage block length per chromosome in the gametes of the first generation individuals would result from the recombinations which would occur within one meiotic division. A segment length will be the measure in genetic map units between two consecutive points at which genetic recombinations had occurred during any generation of intermating. A segment length thus defined measures the length of an initial linkage segment still intact in the nth generation gametes of an intermating population.

The problem can be explicitly stated. After n generations of intermating, determine the distribution which characterizes the length of any one segment occurring within a chromosome of a gamete arising from the population. Given this distribution, one can compute the average length and variance of the linkage segment per chromosome intact in the nth generation gametes. For simplicity of presentation one chromosome of the genome will be considered.

The development of the probability structure for this distribution can be facilitated by a simple partition of the segments in the chromosomes of the gametic array of an intermating population. After n generations of mating, the chromosome of a gamete from the intermating population will be characterized by having a total of x points of genetic recombination for the n generations of intermating. The segment length between any two consecutive breaks represents an intact unit from a progenitor chromosome. Consider the partition of the chromosomes of the gametic array based on the total number of breaks x which has occurred within the chromosome. The partition yields an infinite number of disjoint sets, A_{nx}. A_{nx} represents a class of chromosomes which contains a total of x breaks or points of genetic recombination. Each chromosome of the set will contain $(x+1)$ segments identified by the sequence 0 to γ_1, γ_1 to γ_2, . . . , and γ_x to 1.0 where γ_i represents the locus of a break. Identify these segments with the subsets A_{nx1}, A_{nx2}, . . . , A_{nxr}, . . . , respectively, where $1 \leqslant r \leqslant x+1$. For example, A_{nx2} is an infinite subset containing all possible segments generated between the first and second breaks of chromosomes containing a total of x breaks after n generations of intermating or n meiotic divisions. Thus, for each A_{nx}, $(x+1)$ disjoint subsets have been generated whose union is A_{nx}.

The subsets as defined are disjoint since a segment (ω) can occur in one subset only. However, it should be equally apparent that the existence of the subset A_{nxr} implies the existence of the remaining x subsets of A_{nx} since a pattern of x breaks within a chromosome generates $(x+1)$ segments of total length 1.0, the $(x+1)$ segments being associated in subsets by order of occurrence within the chromosome. The peculiarity of the set structure will not complicate the solution of the problem since we are interested in describing a random variable resulting from

the selection of only one of the $(x+1)$ segments, given a chromosome in the A_{nx} class. Consider any segment (ω). Since the defined sets are disjoint, $\omega\epsilon \sum_{x=0}^{\infty} \sum_{r=1}^{x+1} A_{nxr}$. Thus, a random variable $(Z(\omega))$ defined on A_{nxr} can be expressed as

$$Z = \sum_{x=0}^{\infty} \sum_{r=1}^{x+1} z_{nxr} I(A_{nx}) I(A_{nxr}),$$

where $I(A_{nx})$ is an indicator function of A_{nx}, one if A_{nx} occurs and zero otherwise, and $I(A_{nxr})$ is an indicator function, one if A_{nxr} occurs and zero otherwise, given that A_{nx} has occurred (LOÉVE 1955).

It is interesting that the random variable as defined identifies only the element with a specific subset; however, the existence of an element implies the existence of the associated x elements. The total sum of the lengths of the $x+1$ segments is 1.0, the length of the chromosome. Hence,

$$E[Z] = \sum_{x=0}^{\infty} PA_{nx} \left(\sum_{r=1}^{x+1} z_{nxr} P_{A_{nx}} A_{nxr} \right) = \sum_{x=0}^{\infty} P_{nx} \left(\frac{1}{x+1} \right),$$

as measured on the proportionate scale. $PA_{nx} = P_{nx}$ represents the probability that a chromosome will contain exactly x breaks after n generations of random mating and would be defined by some discrete distribution. Since x breaks within a chromosome generate $(x+1)$ segments, $P_{A_{nx}} A_{nxr} = \left(\frac{1}{x+1} \right)$. It is interesting that in determining the average length of segment per chromosome after n generations of random mating the assumptions made for interference will involve only the distribution of chromosome types (P_{nx}).

Consider next the derivation of the cumulative distribution, $F(t_n)$, which defines the probability that the random variable is less than or equal to some proportion of the chromosome, t. The set $B(t_n)$ is defined:

$$B(t_n) = \bigcup_{x=0}^{\infty} \bigcup_{r=1}^{x+1} \{\omega:\ \omega\epsilon A_{nxr},\ Z(\omega) \leqslant t_n\},$$

which represents the union of disjoint sets. It follows that $F(t_n)$ is the probability that any segment, ω, is an element of $B(t_n)$, and

$$F(t_n) = \sum_{x=0}^{\infty} P_{nx} \left[\frac{1}{x+1} \right] \sum_{r=1}^{x+1} Pr[Z(\omega) \leqslant t_n:\ \omega\epsilon A_{nxr}].$$

Further considerations for determining the cumulative distribution of segment lengths, $F(t_n)$, cannot be made unless an assumption for interference is defined. The assumption will be made that the occurrence of points of genetic recombination are independent in a probability sense. This assumption is not critical as n becomes large. Although interference will tend to restrict the pattern of breaks for any one meiotic division, the breaks occurring in different generations will be independent in a probability sense. With the assumption of independence, the probability P_{1x} that a chromosome resulting from a meiotic division will contain exactly x breaks may be defined by the Poisson distribution, and $P_{1x} = (e^{-s} s^x / x!)$,

where $s = E[x]$ is the length of the genetic map for a chromosome (HANSON 1959). P_{nx} can be expressed in terms of $P_{(n-1)x}$ as follows:

$$P_{nx} = \sum_{r=0}^{x} P_{(n-1)r} P_{1(x-r)}.$$

After n generations of intermating,

$$P_{nx} = [e^{-ns}(ns)^x/x!].$$

The result is expected. If one accumulated the number of breaks which occurred in a chromosome during n meiotic divisions, the result would be equivalent to a chromosome of genetic map length ns.

The procedure for determining the probability that a segment length is less than or equal to some proportionate length t is relatively straightforward. Through the operation of sets, the problem reduces to determining the probability that the random variable is less than or equal to t, given a subset A_{nxr}. With the assumption of independence the locus of the points of genetic recombination, γ_i, are characterized by a uniform density with respect to the genetic map scale. Thus, it can be shown that the joint distribution of the γ_i's when taken in sequence is also uniform. The subset A_{nxr} is generated by the points γ_{r-1} to γ_r. The problem requires the solution of the $Pr(\gamma_r - \gamma_{r-1}) \leqslant t$, where $0 < \gamma_1 < \gamma_2 < \ldots < \gamma_{r-1} < \gamma_r < \ldots < \gamma_x < 1.0$, which is relatively straightforward (HANSON 1959), and

$$Pr[Z(\omega) \leqslant t_n : \omega \epsilon A_{nx1}] = Pr[Z(\omega) \leqslant t_n : \omega \epsilon A_{nx2}] = \cdots = 1 - (1-t_n)^x.$$

Thus,

$$Pr[Z(\omega) \leqslant t_n] = \sum_{x=1}^{\infty} P_{nx}[1-(1-t_n)^x] = 1 - \sum_{0}^{\infty} P_{nx}(1-t_n)^x$$
$$= 1 - e^{-nst_n}, \qquad 0 \leqslant t_n < 1.0,$$

$$Pr[Z(\omega) = 1.0] = e^{-ns}.$$

Since the cumulative distribution of segment lengths ($F(t_n)$) is known,

$$E[Z^m] = \int_0^1 t_n^m dF(t_n) + (1)^m e^{-ns}.$$

$F(t_n)$ is discontinuous at $t_n = 1.0$. Since t_n is measured on the proportionate scale, the segment lengths can be converted to genetic map units by $c_n^m = t_n^m s^m$.

The expected segment length per chromosome and variance of lengths in the

TABLE 1

Expected lengths (centimorgans/100) and variances of initial linkage blocks intact in the gametes of a population intermated for n *generations*

Statistic	Length chromosome (s)	Generations of intermating (n)										
		1	2	3	4	5	6	8	10	12	16	24
Expected segment length per chromosome	0.5	.393	.316	.259	.216	.184	.158	.123	.099	.083	.052	.042
	1.0	.632	.432	.317	.245	.199	.166	.125	.100	.083	.062	.042
	2.0	.865	.491	.333	.250	.200	.167	.125	.100	.083	.062	.042
Expected variance of segment length	0.5	.0256	.0322	.0312	.0275	.0233	.0194	.0133	.0093	.0067	.0039	.0017
	1.0	.1289	.1101	.0776	.0533	.0373	.0270	.0155	.0100	.0069	.0039	.0017
	2.0	.4404	.2133	.1078	.0622	.0400	.0278	.0156	.0100	.0069	.0039	.0017

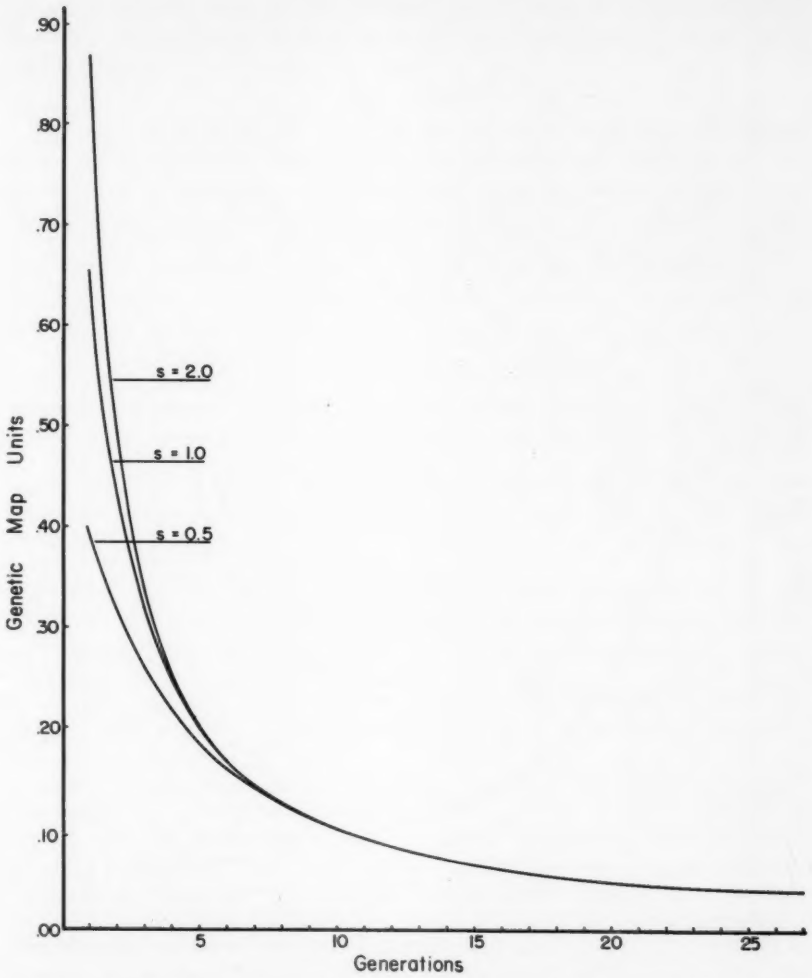

FIGURE 1.—The expected segment length per chromosome (centimorgans/100) in the nth generation gametes of an intermating population, given selected chromosome map lengths.

gametic array of a population after n generations of intermating are given in Table 1 and in Figures 1 and 2 for three selected chromosome lengths, 0.5, 1.0 and 2.0. A chromosome length of 1.0 may be taken as a representative chromosome length. After six to eight generations of mating the distributions of segment lengths for the range of map lengths selected become quite similar. The expected

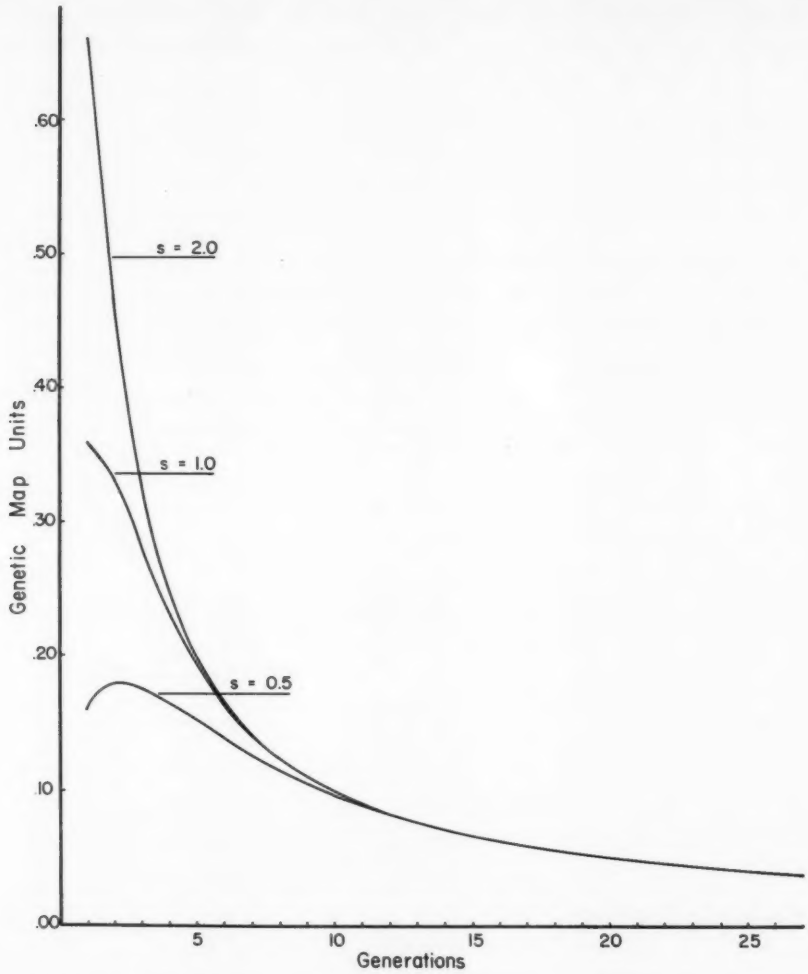

FIGURE 2.—The standard error ($\sqrt{E[V(c)]}$) of segment lengths per chromosome (centimorgans/100) in the nth generation gametes of an intermating population, given selected chromosome map lengths.

segment length and variance of segment lengths approach $1/n$ and $1/n^2$, respectively, in the limit. For a long chromosome, such as $s = 2.0$, the long segments yield skewed distributions during the initial generations of mating with relatively large variances, while for a short chromosome, such as $s = 0.5$, the increase of

variances in the initial generations of mating results from the breakup of the segments near 0.5 in length, which predominate the distribution of first generation segment lengths.

DISCUSSION

The theoretical distribution of lengths of linkage blocks from the initial generation, $n = 0$, intact after n generations of intermating was developed. The restrictions for the development required (i) a population in any generation was of sufficient size so that the probability of a genetic recombination involving linkage blocks from one of the original progenitor chromosomes was small and could be ignored and (ii) the occurrence of any two points of genetic recombination was independent in a probability sense. If restriction (i) holds for all generations of intermating, the results are applicable to a random mating population or to an intermating population in which selection has been practiced. If the number of progenitor chromosomes is small or if the intermating population is of restricted size, then the calculations yield minimum expected lengths, since any point of genetic recombination within a chromosome of a gametic array which involved two identical progenitor chromosome segments will not affect a reduction in length of the initial linkage block. Restriction (ii) yields limitations in the segment analysis in the initial generations of intermating (HANSON 1959). However, as n becomes large, restriction (ii) is not critical since the breaks occurring in two different generations would be independent.

For the segment analysis the unit of measure involved segment length per chromosome, which yields average segment length per chromosome. This concept defined an operation of sets which resolved the problem into relatively easy mathematical manipulations. The cumulative distribution, $F(t_n)$, thus obtained defines the probability that any selected segment within a chromosome obtained from the gametic array of the population after n generations of intermating will be less than or equal to t_n in length.

After approximately eight meiotic divisions, the average segment length and variance of segment lengths were approximately $1/n$ and $1/n^2$, respectively. However, the average segment length per chromosome would be dependent upon the genetic map length of the chromosome in the initial generations of intermating. The primary breakup of relatively long linkage blocks would occur in the first four or five generations of intermating. However, the average segment length per chromosome would still be 0.20 to 0.25 map units (or 20 to 25 centimorgans) in length.

SUMMARY

The cumulative distribution for the lengths of linkage blocks intact after n meiotic divisions was developed for an intermating population within which the probability that a recombination will involve a locus heterozygous for a progenitor chromosome type was small and could be ignored. The average segment length per chromosome, with respect to the genetic map scale, and the variance of segment lengths were given for selected chromosome map lengths. The princi-

pal reduction in the average segment lengths occurred in the first 4–5 generations of intermating. After about eight meiotic divisions the average segment lengths and the variances of segment lengths were essentially the same for the chromosome map lengths selected and could be approximated by the limiting forms of $1/n$ and $1/n^2$, respectively.

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DETAILED ANALYSIS OF A NEUROSPORA CROSS

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ACCORDING to a widely accepted view of the mechanism of ascospore formation in *Neurospora crassa*, a single diploid nucleus in the young ascus undergoes meiosis followed by mitotic division of the four resulting nuclei. A spore is formed around each of the eight products, sister nuclei from the mitotic divisions inhabiting adjacent spores. A second mitosis gives binucleate spores. This mechanism leads to the following expectations: that the nuclei within a spore are haploid and genetically alike; that the members of a spore pair are genetically identical and, therefore, that not more than four phenotypes will be found in one ascus; that each ascus represents an independent event.

Occasional exceptions to the first expectation, that is, single-ascospore cultures which were obviously heterocaryotic (PITTENGER 1954) have been thought to result from irregular meioses and, hence, to occur in abnormal asci. There are indications, however, that certain types of heterocaryons, difficult to recognize, may not be noticed ordinarily and that the frequency of exceptions of this sort is unknown.

It is also difficult to estimate, from existing data, the frequency of spore pairs whose members are not alike phenotypically. In some analyses homogeneity has been taken for granted and only one member of a pair tested (segregation of mating type in 1814 asci, HOULAHAN, BEADLE and CALHOUN 1949) or the spores have been isolated and cultured as pairs. Among completely germinated and completely scored asci about one in 20 shows irregularities in the order of phenotypes. Instead of the order 1,1,2,2,3,3,4,4, one may find, for example, 1,2,2,1 or 1,2,1,2, and so forth. Less frequently five to three segregations have been recorded. Among 1878 asci (HOULAHAN, BEADLE and CALHOUN 1949), of which 814 germinated completely and were completely scored, 11 were recorded as five to three segregations. These irregularities have been attributed to errors in technique, although, in some of the latter cases, this seems a bit unlikely.

A recent observation appears to be contrary to the expectation that the formation of each ascus represents an independent event. By examination of immature perithecia, asci were frequently seen to be formed in pairs which are closely associated and appear to be at exactly the same stage of development. The striking similarity of these immature twin asci suggests the possibility that they may resemble one another in genetic constitution.

The work reported here constitutes an attempt to detect twin phenotypic patterns and also to estimate the frequency of heterocaryotic spores and heterogeneous spore pairs in a cross analyzed for that purpose. Hence, in choosing the

cross described the considerations were clarity and simplicity of classification of its progeny rather than suitability of the parents for enzymatic or biochemical studies. The analyses have been done with particular care so as to eliminate, in so far as possible, errors in technique which might serve to explain away irregularities.

Phenotypes

The cross to be considered is of two unlinked colonial mutants, C 102, *cot*, temperature-sensitive colonial and C 136, *sn*, snowflake (MITCHELL 1958). Snowflake shows close linkage with nutritional mutants assigned to positions near the centromere in linkage group I, whereas *cot* exhibits linkage to *pan-1* in group IV. Two descendants of each mutant from crosses to standard wilds were used. Stock cultures of these strains have been maintained on unsupplemented WESTERGAARD and MITCHELL (1947) medium.

The *sn* × *cot* cross was chosen for the present purpose partly because the segregants can be scored on minimal plates within 20 hours after germination. It is then possible to observe in detail the first hyphae which grow from the spore and to see clearly that each colony scored arises from a single ascospore. The minimal medium used is a modified Fries in which ammonium tartrate and ammonium nitrate are replaced by the corresponding potassium salts (STRAUSS 1951). After 15 hours incubation at 25°C two types, *sn* and *sn*$^+$, can be scored. At this temperature *cot* is not distinguishable from *sn*$^+$ *cot*$^+$ nor *sn cot* from *sn*. When the temperature is shifted to 34°C, elongation of the hyphae of *cot* and *sn cot* stops abruptly and, during the next three hours, short branches form at rather regular intervals along the whole length of the hyphae. When the segregants are again incubated at 25°C for half an hour elongation of the hyphae is resumed and the short branches formed at 34°C elongate rapidly, so that the *cot* character becomes more conspicuous.

In addition to *sn*, *cot*, *sn cot* and *sn*$^+$ *cot*$^+$ (designated here as + since only the phenotypes are being considered) it was possible to score two "modified" forms of *cot*, designated as *dcot*, dilute, and *ddcot*, dilute dilute, and one variegated form, designated as *vcot*. Variegated *cot* was not frequent in this cross but it is quite interesting since the simplest interpretation of it is in terms of genetic heterogeneity of single spores. At the time of scoring, *cot* and + colonies show several hyphae, around five or six, as branches of the original short germ tubes. In *vcot* one or two of these hyphae expressed "typical" *cot* throughout their length while the remaining hyphae were typically +. The latter continued elongation at 34°C and branched infrequently in the manner characteristic of +. The *dcot* form appears homogeneous and, like typical *cot*, ceases elongation at 34°C but it differs in that the branches formed are shorter and less frequent. Hyphae of the remaining type, *ddcot* do not elongate at 34°C and form few branches which are very short and appear near the ends. The term, dilute, describes the appearance of the colonies but, in so far as the temperature effect is concerned, the dilute forms may be the more extreme ones. They show less growth, fewer and shorter branches, at 34°C.

A curious attribute of *sn* is the phenotypic shift in the direction of + which it regularly undergoes. The initial growth is colonial in character, that is, the hyphae show excessive branching, but this decreases as growth continues and, in a slant culture, the final growth is almost normal. Colonies from single ascospores or conidia on plates show sectoring but even the less extreme sectors are usually quite distinct from sn^+ for several days.

At the time of scoring *sn* colonies are small and consist of a single layer or network of hyphae in which individual branches of the original germ tubes can often be followed. Like *cot*, *sn* was variable in expression but there were more *sn* classes, less distinct from one another; therefore no attempt was made to score them separately. The *sn cot* colonies do not enlarge at 34°C, but become more dense, as the *cot* branching is superimposed on the frequent branches already formed. A *sn cot* colony is distinguished from an extreme *sn* by the uniformity in length and spacing of the *cot* branches. It seems likely, however, that *sn vcot* would be scored as *sn* and that *sn ddcot* might also be so scored. However, in completely germinated asci, *sn* and *sn cot* spore pairs appeared quite homogeneous. It will be seen that in only one case was there observed a convincing difference which could not be accounted for in terms of mistakes in recording spore order.

Random spores

Crosses were made on slants of WESTERGAARD and MITCHELL medium by placing inocula from the two parents together on the slant and mixing them. After 17 days or more, samples of spontaneously released ascospores were suspended in about 0.5 ml of sterile water. The suspensions were spread on plates containing the modified Fries medium solidified with four percent agar. After heat treatment at 60°C for ½ hour the plates were incubated, first at 25°C for 15 hours, then at 34°C for three hours and again at 25°C for ½ hour. Incubation at 34°C was sometimes omitted for *sn* × *sn* crosses.

The cross of the two *sn* isolates used here gave mainly *sn* progeny. About 15 to 20 percent of the normal-appearing spores failed to germinate and one or two percent formed colonies which grew too little to be scored after 20 hours. No typical + colonies were observed but it should be mentioned that crosses of these isolates to *sn* segregants from certain other crosses have been observed to give typical + progeny and also slow growing sn^+ segregants.

Typical + progeny have not been found from *cot* × *cot*, but about one percent behaved as auxotrophs in that they formed short germ tubes which did not continue to grow. About 0.5 percent of the progeny showed a colonial character at 25°C and 2.3 percent among 1350 germinated spores were classified as *dcot* or *ddcot*. Rather large scale tests have been performed incidental to the use of *cot* × *cot* crosses to introduce colonial growth at 31°C in material examined for new mutants. Typical + offspring were not found even though one parent was treated with a mutagen, usually ultraviolet light. For this reason *cot* is regarded as an unusually stable mutant.

The cross *sn A* × *cot a* was somewhat more fertile than either of the "selfs,"

and germination of spontaneously released black spores was usually above 98 percent. Spores were plated repeatedly in order to work out the best conditions for classification. A series of four plates, incubated in the manner described above, gave the following frequencies among 2553 spores: *sn*, 25.1; *sn cot*, 24.4; *cot*, 20.8; +, 29.6, all in percent. The four forms of *cot* were counted together. All plates contained a few segregants which had grown so little that they could not be classified.

The cross *sn a* × *cot A* gave about the same fertility and germination, but scoring was more difficult. The variability of *sn* was greater and "small," unclassifiable individuals more numerous. The following frequencies were obtained from two plates containing 1515 spores; *sn*, 24.3; *sn cot*, 27.3; *cot*, 21.3; +, 27.1 percent.

Segregation in asci

Asci were dissected on the minimum plates; the spores were placed at suitable distances apart in rows and all unused material was removed. In order to ensure that whole asci were being examined two checks were made. A group of asci still attached to the cluster was chosen and watched while a small drop of water was placed on the cluster. The asci float in water and an intact ascus floats as a unit. The water was allowed to evaporate and the ascus to be dissected was stretched with a glass needle placed between the first and second spores. All the spores of an intact ascus move in response to this pull and then move back in the direction of their former positions when the ascus is released.

The two more frequent types of asci from *sn A* × *cot a* after about 17 days incubation were those with eight black spores or with eight colorless spores. Asci in which no spores had formed were also numerous. One hundred and fifty-five asci containing black spores were dissected. Most of these had eight spores but a few had only six or seven. Only the 109 eight-spored asci from which all spores germinated will be considered here, except to say that the more frequent reason for failure to germinate may have been immaturity. This is suggested by the observation that samples of spontaneously released black spores regularly gave almost perfect germination.

Tabulations of the asci, in Tables 1 and 2, show the order in which the spores were isolated as well as the phenotypes of the segregants. The asci in Table 1 contained only the four phenotypes, *sn*, *sn cot*, *cot* and + and were regular except for the last four which show irregularities only in the order of phenotypes. Table 2 shows all other asci with heterogeneous spore pairs and also six with homogeneous pairs but which contained one of the "atypical" forms of *cot*.

In the first eight irregular asci the heterogeneous spore pair consists of + and one of the three *cot* types, *cot*, *vcot* or *dcot*. These + segregants were indistinguishable from + individuals in regular asci. The heterogeneous pair in the next 11 asci consists of *cot* with one of its "modified" forms. It is considered unlikely that the heterogeneity here is due to chance environmental or physiological differences not reflecting differences in genetic constitution of the hyphae. Envi-

TABLE 1

Regular segregations from sn A × cot a

1*	2	3	4	5	6	7	8	Number of asci
sn	*sn*	*sn*	*sn*	*cot*	*cot*	*cot*	*cot*	9
cot	*cot*	*cot*	*cot*	*sn*	*sn*	*sn*	*sn*	7
sn cot	*sn cot*	*sn cot*	*sn cot*	+	+	+	+	9
+	+	+	+	*sn cot*	*sn cot*	*sn cot*	*sn cot*	16
sn cot	*sn cot*	*sn*	*sn*	*cot*	*cot*	+	+	4
+	+	*cot*	*cot*	*sn*	*sn*	*sn cot*	*sn cot*	7
sn cot	*sn cot*	*sn*	*sn*	+	+	*cot*	*cot*	6
+	+	*cot*	*cot*	*sn cot*	*sn cot*	*sn*	*sn*	3
sn	*sn*	*sn cot*	*sn cot*	*cot*	*cot*	+	+	5
cot	*cot*	+	+	*sn*	*sn*	*sn cot*	*sn cot*	6
sn	*sn*	*sn cot*	*sn cot*	+	+	*cot*	*cot*	2
cot	*cot*	+	+	*sn cot*	*sn cot*	*sn*	*sn*	6
+	+	+	*sn cot*	+	*sn cot*	*sn cot*	*sn cot*	1
sn	*sn cot*	*sn*	*sn cot*	+	+	*cot*	*cot*	1
sn cot	*sn*	*sn cot*	*sn*	+	+	*cot*	*cot*	1
cot	+	*cot*	+	*sn*	*sn*	*sn cot*	*sn cot*	1
								84

* Each line shows the order of spores from 1 to 8 in an ascus sac.

ronmental effects seem an unlikely explanation because, in each case, the two segregants were growing a few mm apart on the same plate. Chance physiological differences would not account for the pattern that is followed among the *sn*+ segregants. It will be seen that no ascus contains more than two of the *cot* forms and, when there are two, one of them is always "typical" *cot*. No effort was made at the time of scoring to fit the asci into this pattern. It was not noticed until after the analysis was completed.

It seems possible, however, that, in some cases at least, the members of the heterogeneous spore pairs were originally alike genetically but both were heterocaryotic. Chance factors affecting multiplication of the components of the heterocaryon might then account for the difference in expression in the two segregants. This seems particularly applicable to those pairs having *vcot* as one member, since this type appears as a heterogeneous colony.

Twin asci

The twin asci were seen in preparations of immature asci fixed in ethanol-acetic acid and stained lightly with aceto-carmine or aceto-lacmoid. Examinations were made with phase contrast which makes it possible to see structural details not visible in ordinary light. The twins are attached to the ascus cluster

TABLE 2

Irregular segregations from sn A × cot a

1*	2	3	4	5	6	7	8	Number of asci
sn cot	*sn cot*	*sn*	*sn*	+	+	+	*cot*	1
sn	*sn*	*sn cot*	*sn cot*	*cot*	+	+	+	1
sn cot	*sn cot*	*sn*	*sn*	+	*cot*	+	+	1
sn	*sn*	*sn cot*	*sn cot*	+	+	*vcot*†	+	1
+	+	+	*vcot*	*sn*	*sn*	*sn cot*	*sn cot*	1
sn	*sn*	*sn*	*sn*	*dcot*‡	*dcot*	*dcot*	+	1
sn	*sn*	*sn cot*	*sn cot*	*dcot*	+	+	+	1
sn cot	*sn cot*	*sn*	*sn*	+	+	*dcot*	+	1
sn	*sn*	*sn cot*	*cot*	*sn cot*	*vcot*	+	+	1
sn	*sn*	*sn*	*sn*	*cot*	*dcot*	*cot*	*cot*	1
cot	*cot*	*cot*	*dcot*	*sn*	*sn*	*sn*	*sn*	1
dcot	*dcot*	*cot*	*dcot*	*sn*	*sn*	*sn*	*sn*	1
+	+	+	+	*sn dcot*	*sn cot*	*sn cot*	*sn cot*	1
sn	*sn*	*sn cot*	*sn cot*	+	+	*dcot*	*cot*	2
+	+	*cot*	*dcot*	*sn*	*sn*	*sn cot*	*sn cot*	1
cot	*dcot*	+	+	*sn*	*sn*	*sn cot*	*sn cot*	1
cot	*cot*	*ddcot*	*cot*	*sn*	*sn*	*sn*	*sn*	1
sn	*sn*	*sn cot*	*sn cot*	*cot*	*ddcot*	+	+	1
sn	*sn*	*sn*	*sn*	*dcot*	*dcot*	*cot*	*cot*	2
cot	*cot*	*dcot*	*dcot*	*sn*	*sn*	*sn*	*sn*	1
sn	*sn*	*sn cot*	*sn cot*	+	+	*dcot*	*dcot*	1
dcot	*dcot*	+	+	*sn*	*sn*	*sn cot*	*sn cot*	1
sn cot	*sn cot*	*sn*	*sn*	*ddcot*	*ddcot*	+	+	1
								—
								25

* Each line shows the order of spores from 1 to 8 in an ascus sac.
† *vcot* = variegated *cot*.
‡ *dcot* and *ddcot* = modified forms of *cot*.

at adjacent points and appear almost identical in such details as size, shape, degenerate areas in the cytoplasm, the appearance of the apical pore, the number and appearance of nuclei or the number, shape, size and degree of maturity or degeneration of spores (Figure 1). Some details regarding association of the twins during development will be omitted here. It appears that the actual process of ascus formation is an intricate one and it is hoped that after further study this process can be described more completely than would be possible now.

In order to be sure that adjacent mature asci are twins it would be necessary to see details which are not visible with the magnification used for dissection. With the equipment available, all that could be done was to dissect adjacent asci whenever possible and to record their positions. One pattern of twin phenotypes which is suggested by the associations thus observed is set forth in Table 1, the

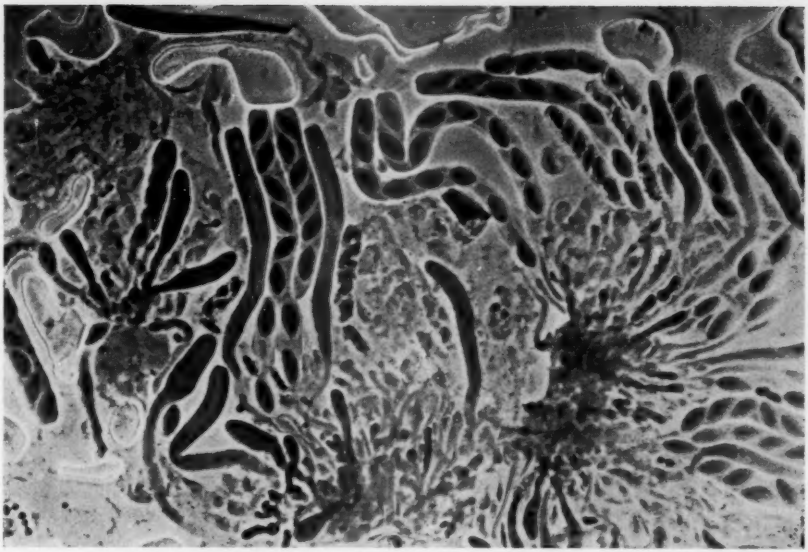

FIGURE 1.—Immature or degenerate twin asci from a typical cross.

first and second ascus types (the two parental-ditype arrangements) being twins, the third and fourth, and so on. This pattern seems more likely than one which makes the twins identical in both arrangement and phenotype. It is an interesting possibility, partly because it would, presumably, fit all crosses as well as would the identical pattern, and partly because of the suggested relationship between corresponding spore pairs in twin asci. There is, however, no assurance at present that the same pattern will be found in all crosses, nor is it certain that all asci are formed in pairs. Indeed, one recognizable type, seen more frequently in certain other crosses than in this one, appears not to have a twin.

One other point might be mentioned in favor of the suggested pattern, namely that it would, in part, account for odd coincidences sometimes found. Data from a cross of the linked mutants, *pan-1* and *pdx* (HOULAHAN, BEADLE and CALHOUN 1949) will serve as an illustration. Twenty-one among 49 asci were recombinant and, in terms of crossovers, 17 of these can be accounted for as singles between *pdx* and *pan*. Of the four requiring double crossovers, two were as follows:

pan	*pan*	*pdx*	*pdx*	+	+	*pdx*	*pan*	*pdx*	*pan*
−	*pdx*	*pan*	*pan*	−	*pdx*	*pan*	+		+

Seven perithecia were analyzed and these two asci, the only representatives of this type among 49, were obtained from the same perithecium. Thus it is possible that they were twins.

DISCUSSION

In earlier analyses only 11 of 814 asci were recorded as five to three segregations whereas, in the present study 19 among 109 were found to contain heterogeneous spore pairs. One may well ask whether this difference is due to differences in the strains, in the methods, or in the attitude of the observer. It is the author's opinion that all three factors may have played a part. Certainly it appears that some phenotypes segregate more irregularly than others, at least in some crosses. This is indicated by the fact that all irregularities here (disregarding irregularities in order only) involve *cot*, while segregation of *sn* was recorded as regular in each ascus. As for methods, the ones used here and the properties of *cot* make it possible to detect slight differences in phenotype which would have been unnoticed or ignored in the earlier work. If, for example, *cot* were a nutritional mutant, scored by the earlier method of establishing single-spore cultures on complete and sub-culturing on minimal, such differences as those between *cot*, *dcot* and *ddcot* would have been unnoticed or attributed to environmental effects. Thus ten of the heterogeneous spore pairs would not have been recorded. The attitude of the observer, at the time of the earlier work, may well have been influenced by the conviction that the two members of a spore pair were genetically identical. During the present study a more neutral point of view has been entertained.

Because of recent interest in six to two segregations in Neurospora (MITCHELL 1956; CASE and GILES 1958) it may be worth while to emphasize the fact that no unambiguous six to two segregations were recorded here. It appears, however, as was suggested above, that the distinction between six to two and five to three segregation may be an artificial one. Two asci (the fourth and fifth in Table 2) would most likely have been scored as six to two segregations if *cot* were a nutritional mutant. The heterogeneous pair consists of + and *vcot* and, even by the method used here, such a variegated form of a nutritional mutant would have been scored as +. The mutant hyphal branches would merely have stopped growing very early and the presence of one or two short branches would have been disregarded since this is commonly seen in + colonies. Nor does it seem assured that subsequent tests would reveal the heterogeneous nature of such variegated segregants, even if the initial growth occurred on supplemented medium. It is well known that growth of many nutritional mutants is slower than that of +, even on supplemented medium. The mutant component might, therefore, have become so outnumbered by + as to make its recovery from an outcross unlikely.

Testing the segregants of irregular asci by outcrossing seems less to the point here than in the case of six to two segregation. The finding that a pair of *cot*$^+$ segregants, from a six *cot*$^+$ to two *cot* ascus, behaved in outcrosses as suppressed *cot* could be regarded as an explanation of the irregular segregation. In an ascus such as the first one in Table 2, however, the more interesting question is not why the seventh segregant (assuming the spore order to have been recorded cor-

rectly) is wild, but why it is phenotypically different from the eight. The latter question would not, of course, be answered by the finding that number seven was suppressed *cot*, which, moreover, does not seem very likely. When the same phenotypic difference, that between + and *cot*, is observed in a regular ascus it is assumed, without further tests, to represent a genetic difference. In the absence of prior convictions, it therefore seems reasonable to assume a genetic difference between the seventh and eighth segregants in the irregular ascus, particularly when it is remembered that the genetic identity of members of a spore pair has not been demonstrated. They have been assumed to be regularly alike genetically, partly because they so often appear to be phenotypically alike. Hence it was decided to forego the outcrosses and to pursue instead, as a continuation of this study, such considerations as the possible correlation suggested here between five to three segregation of phenotypes and the ability to segregate "at the second division".

The phenotypic shift towards + regularly exhibited by *sn* cultures is of interest because this sort of "adaptation" is characteristic of many single-ascospore strains of Neurospora. Certain growth responses, for example, have been described as "adaptive" when the growth rate is slow initially but becomes more rapid as the culture ages. A possible interpretation of this behavior can be made in terms of genetic components which give different expressions and which are capable of independent multiplication. If neither alone could produce a viable mycelium, then, together they might constitute an obligate heterocaryon whose phenotype could shift within certain limits in response to culture conditions. Deficient components of heterocaryons were suggested earlier to account for the behavior of certain pseudo wilds (MITCHELL, PITTENGER and MITCHELL 1952). In the present case it seems premature to attempt to devise an explanation of the regular appearance of different and independently multiplying components from single spores of normal eight-spored asci. The fact that *vcot* spores, obviously heterogeneous, were obtained from such asci, suggests, however, that it may be useful to keep such a possibility in mind.

It may be seen that of 19 asci in which the "modified" forms, *dcot* and *ddcot*, appeared, 13 were irregular, whereas only six among 90 not containing these forms showed the same sort of irregularity. This indicates a correlation between irregular segregation and "modification". If *cot* is regarded as a depressor of growth at 34°C, then *dcot* becomes an enhanced form of *cot* and *ddcot* an enhanced *dcot* or, perhaps, a doubly enhanced *cot*. This suggests the possibility that the enhancer is *cot* itself, *dcot* then being *cot cot* and *ddcot*, *cot cot cot*. Possibly, on some basis, the frequencies of the three types among the *sn*+ segregants from asci would then be as expected. The parent type, *cot*, is about eight times as frequent as *dcot* which is about six times as frequent as *ddcot*. The same basis might also provide reason to expect a correlation between "modification" and the occurrence of heterogeneous spore pairs.

SUMMARY

A cross of two unlinked colonial mutants has been carefully analyzed, with particular attention given to segregations in asci. The four phenotypes expected, two parental and two recombinant, and also one variegated and two "modified" forms of one parent, could be classified as very young colonies which could be seen to have originated from single ascospores germinated on minimal plates.

All completely germinated asci (109) showed four to four segregation of one of the mutants. Nineteen of these asci contained heterogeneous spore pairs which could not be accounted for in terms of mistaken spore order. The heterogeneous pairs consisted of the second mutant and wild, or one of its atypical forms and wild, or the typical with an atypical form. Thus six asci contained five phenotypes instead of the expected maximum of four. Three of the asci contained one spore each which gave rise to a heterogeneous colony, consisting of the typical mutant form and wild. Curiously, the mutant implicated in the irregularities is one which, in other tests, has shown exceptional stability. The regularly segregating mutant, on the other hand, has been observed to give nonmutant offspring from crosses to certain descendants of itself. No detailed interpretation of the irregularities is offered. The possible prevalence of genetically heterogeneous spores is considered.

Observations of immature perithecia have shown that asci develop as closely associated pairs, or twins. The twins are so nearly identical with respect to structural details as to suggest that they may be identical in phenotype. Twin phenotypes, suggested by the ascus analyses, are discussed.

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THE BREAKUP OF INITIAL LINKAGE BLOCKS UNDER SELECTED MATING SYSTEMS

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THE extent to which linkage blocks are broken and to which recombination occurs is of critical interest to the plant breeder since the success of his breeding program depends upon obtaining desirable genetic recombinations. The conventional procedure for the improvement of a self-pollinated species involves a program of continued crossing and pure lining. If the genetic diversity can be maintained in the breeding material, effective breakup of linkage blocks and recombination of genetic types will be achieved over an extended period of time. Unfortunately, genetic diversity is not always maintained in the advanced breeding stock. Information on the extent to which linkage blocks can be broken if a gene pool were held in a heterozygous state for a selected number of generations and then pure lined would be extremely helpful in evaluating breeding procedures. The objective of this paper is to derive the average lengths of linkage blocks per chromosome which are intact after selected breeding procedures and to interpret the information available from a segment analysis. Mating systems involving random mating within a population synthesized from a limited number of parents and pure lining from such a random mating population will be considered in detail in this paper.

Derivation of average segment length per chromosome

Segment lengths will be considered with reference to the true genetic map scale. The true genetic map distance for a segment length is the expected number of breaks or points of genetic recombination for the segment (FISHER 1948). A genetic map length for a chromosome will be taken as s. The length of any segment will be defined as c, $0 \leqslant c \leqslant s$. The transformation $t = c/s$ places all measurements on a proportionate scale. Thus, a chromosome length is 1.0 and $0 \leqslant t \leqslant 1.0$. The proportionate scale will be used in the derivations which follow.

Linkage blocks will be considered intact with reference to the original progenitor chromosome types contributed by each parent in a mating system. If one considers the chromosomes resulting from a meiotic division, a reduction in linkage block length will be effected only if the locus of a point of recombination is heterozygous with respect to progenitor chromosome types. Although a recombination involving a locus homozygous for a progenitor chromosome may cause a reshuffling of breaks from previous divisions, the average effect in the gametic array is the same as if the recombination had not occurred. The length of a

chromosome segment which represents an intact linkage unit from a progenitor chromosome is the map length between two consecutive loci of effective recombinations, or recombinations involving loci heterozygous for two progenitor chromosome types.

If one were to develop the expected segment lengths for a mating system by considering the mechanics of a meiotic division, the problem would be extremely complex. The problem is appreciably simplified if the products of the meiotic divisions, or the gametic arrays, are considered. HANSON (1959a) has developed the distribution of segment lengths in the gametes of an F_1 individual and (1959b) has extended the concept to include the distribution of segment lengths in the gametes of a population intermated for n generations. In the latter publication the assumption was made that the probability of a recombination involving a locus homozygous for a progenitor chromosome type was small and could be ignored. Theoretical ramifications for the distribution of segment lengths were given in detail. The approach involved the description for the discrete distribution of chromosomes having a total of x breaks or points of genetic recombination after n meiotic divisions. Given the probability (P_{nx}) that a chromosome will have a total of x breaks, the average proportionate segment length per chromosome can be computed, $E[t_n] = \sum_{x=0}^{\infty} P_{nx}[\frac{1}{x+1}]$. With the assumption of independence (no interference),

$$P_{nx} = e^{-ns}(ns)^x/x!. \qquad \text{(i)}$$

In this paper the assumption will be made that the occurrence of any two breaks is independent in a probability sense. The limitations imposed by this assumption are not as critical as might be expected. The assumption of independence did not appreciably affect the estimate of average segment length per chromosome in the gametes from an F_1 individual (HANSON 1959a). Since only a limited number of recombinations will have occurred within a chromosome in any one meiotic division and breaks will occur independently between generations, the assumption of independence will not be critical when the distribution of the total number of breaks after n meiotic divisions is considered.

The distribution for the total number of breaks or points of genetic recombination occurring within any chromosome of the gametic array of the population is identical for any mating system, and P_{nx} is defined in (i). However, some of the recombinations will involve loci homozygous for a progenitor chromosome type and will not represent a reduction in the initial linkage block. The probability that a break will involve a locus heterozygous for progenitor chromosome types will depend upon the mating system. The solution requires a description for the distribution of x',the number of effective breaks per chromosome. If $P_{nx'}$ represents the probability of obtaining a chromosome with x' effective breaks after n meiotic divisions, then

$$E[t_n] = \sum_{x'=0}^{\infty} P_{nx'}[\frac{1}{x'+1}]. \qquad \text{(ii)}$$

The solution for $P_{nx'}$ requires the development of four basic concepts.

(A) *If the total proportionate length of heterozygous segments for a homologous chromosome pair is* t, *then* $P_{x'}$, *the probability of obtaining* x′ *effective breaks per chromosome in the gametic array, is* $e^{-(ts)}(ts)^{x'}/x'!$. Let t be the total proportionate length of a chromosome pair which is heterozygous for progenitor chromosome types. Since t is the proportion of the genetic map length for a chromosome pair which is heterozygous, the probability that a break will involve a locus within a heterozygous segment and thus represent an effective break is t. Consider the chromosomes of the gametic array from such an individual which contains exactly x breaks. The distribution of effective breaks (x') would be

$$\sum_{x'=0}^{x} \binom{x}{x'} (1-t)^{(x-x')} (t)^{x'}.$$

However, the probability of obtaining x breaks is $P_x = e^{-s}s^x/x!$. Collecting on any x', one obtains

$$\begin{aligned} P_{x'} &= e^{-s}\Big[\sum_{x=x'}^{\infty} \binom{x}{x'} (1-t)^{x-x'} (t)^{x'} (s)^x/x!\Big] \\ &= e^{-ts}(ts)^{x'}/x'!. \end{aligned} \qquad \text{(iii)}$$

(B) *For a mating population the probability that a break will involve a heterozygous segment is the probability that any one locus will be heterozygous for two progenitor types.* The detailed ramifications for this proposition are involved for the general case. The proposition can be shown to hold for the simpler cases where an intermating population is restricted to two, three, or four progenitor chromosome types and, of course, when the number of progenitor chromosome types is large. For illustrative purposes consider the gametic array of a random mating population arising from the cross between two homozygous parents or from an F_2 population. The individuals of the population can be grouped, based on the type of gametes from which they arose. Consider as a group all individuals which resulted from the union of two gametes containing u and v effective breaks, respectively, with a probability P_{uv} of occurring. Since only two progenitor block types need be considered, $(u+v+1)$ segments are involved per chromosome pair, and the average total heterozygous length per chromosome pair for the group is $a_i = E[t] = 1/2$, which holds whether $(u+v)$ is odd or even. If $(u+v)$ is even, $(u+v)/2$ or $(u+v+2)/2$ heterozygous segments are formed regardless of the order of breaks for the pair of homologous chromosomes except for the trivial case. The two types occur in equal frequencies. Similarly, $(u+v+1)/2$ heterozygous segments are always formed if $(u+v)$ is odd. Thus, for the random mating population with two basic progenitor chromosome types or for an F_2 population the average total heterozygous segment length per chromosome pair is 1/2. For three progenitor chromosome types, the pattern of development is identical to that illustrated; however, it is more complex since a sequence of three block types must be considered. The average total heterozygous segment length per chromosome for the population can be shown to be 2/3. For a random mating population from p parents, $a_i = \dfrac{p-1}{p}$ follows by induction. The probability that a break

will involve a heterozygous segment, and thus produce an effective break, is the average total proportionate heterozygous length per chromosome for a generation of mating. The average total proportionate heterozygous length per chromosome is identical to the probability that any one locus will be heterozygous for a mating generation.

(C) *After* n *meiotic divisions, the distribution of effective breaks per chromosome* (x′) *for the* n*th meiotic division can be approximated by considering* a_i, *the probability that a break involved a heterozygous segment, and the approximate distribution approaches the exact distribution for* x′ *as* n *becomes large.* Consider the individuals of the nth generation of breeding. Any individual of the population will be characterized by having a total length of heterozygous segments of t where $E[t] = a_i$, and the central moments of t are μ_r. From (B) a_i also represents the probability that any one locus will be heterozygous in the nth generation of breeding. An individual with a heterozygous length of t will generate a distribution of effective breaks as defined in (iii). Expanding e for each t and considering expectations, one can obtain the following generalization:

$$P_{x'} = e^{-a_i s}(a_i s)^{x'}/x'! + \{ \pm \mu_r\{ f(a_i s)\}\}. \qquad \text{(iv)}$$

The correction series, $\{ \pm \mu_r\{ f(a_i s)\}\}$, is complex and does not warrant a detailed examination here except that the series represents a convergent sequence of convergent sequences and is thus a convergent sequence. $\{f(a_i s)\}$ represents convergent sequences involving $(a_i s)$ and μ_r is small for any r since $0 \leqslant t \leqslant 1.0$. For a random mating population from p parents, the total heterozygous segment length for a chromosome pair of any individual in the nth generation would rapidly approach $a_i = \dfrac{p\text{-}1}{p}$ as n becomes large, and the rth moments of t about a_i will approach zero. It follows from the correction in (iv) or from logic that as n becomes large the distribution of x' approaches

$$P_{x'} = e^{-a_i s}(a_i s)^{x'}/x'!. \qquad \text{(v)}$$

The limiting distribution for x' in a population which has been selfed can be more easily identified from the distribution of total x' which will be considered next. Although (v) represents an approximate distribution of x', the relation is the limiting distribution of x' as n becomes large.

(D) *The distribution of total effective breaks* (x′) *following a selected breeding procedure can be obtained by considering a length of chromosome* (s′) *equivalent to a linkage system which would generate the distribution of effective breaks in one meiotic division.* The distribution for the total number of recombinations per chromosome which has occurred in n meiotic divisions has been defined in (i). Consider t as the total proportionate length of heterozygous segments per chromosome pair for any individual of the n generations and $1/n$ as the probability that any individual was a member of the ith generation. Let $a = E[t]$. a represents the probability in the nth generation that a break occurring in one of the n generations will be an effective break. Following the algebraic

manipulations as outlined in (A) and the ramifications discussed in (C), one obtains the limiting distribution

$$P_{nx'} = e^{-nas}(nas)^{x'}/x'!, \qquad \text{(vi)}$$

for the distribution of total effective breaks per chromosome after n meiotic divisions. The probability that a break generated in the ith generation is an effective break has been defined in (C) as a_i. Since an effective break can arise by any one of n mutually exclusive ways, the probability that any break produced in n meiotic divisions will involve a heterozygous segment is

$$a = \frac{1}{n}\sum_{i=1}^{n} a_i.$$

Substituting for a in (vi), one obtains

$$P_{nx'} = e^{-(s\Sigma a_i)}(s\Sigma a_i)^{x'}/x'!. \qquad \text{(vii)}$$

Thus, equivalent chromosome length can be considered,

$$s' = s\sum_{i=1}^{n} a_i. \qquad \text{(viii)}$$

That is, the contribution of any meiotic division in a breeding plan to the equivalent chromosome length is sa_i, where a_i can be taken as the probability that a locus is heterozygous for progenitor types within a specific division. The expected x' as determined from (vii) is s'. Thus, the distribution of the total effective breaks generated in n meiotic divisions can be determined by considering the distribution of breaks generated in one meiotic division with an equivalent chromosome length of s'.

The limiting distribution of x' for a selfed population can be readily verified as (vi). As n becomes large, $a \to 2/n \to 0$, and all central moments would approach zero. Also, it should be pointed out that $E[t]$ for any division is a_i; hence, the approximation in formula (iv) involves only the final distribution. On the other hand, if the approximation had involved each division, errors would have been compounded, and (vii) would have had limited value.

The average proportionate segment length per chromosome of the progenitor linkage blocks intact in the nth generation gametes of individuals in a breeding system is defined in (ii). Substituting for $P_{nx'}$, one obtains

$$E[c] = sE[t_n] = s[1\text{-}e^{-s'}]/s', \qquad \text{(ix)}$$

where s', the equivalent chromosome length, is defined in (viii) and c is the segment length expressed in terms of the true genetic map scale. The average number of segments per chromosome is

$$E[x'+1] = \sum_{x=0}^{\infty} P_{nx'}(x'+1) = (s'+1). \qquad \text{(x)}$$

Breakup of linkage blocks in a random mating population with a restricted number of progenitor chromosome types

Consider a random mating population synthesized by intermating p homozygous parents. The population will contain p progenitor chromosome types. Assume that each mating produces an equal number of progenies and that the population size is of sufficient magnitude so that genetic drift does not occur. In

this paper $p \geqslant 2$ will be considered. Since p is small, the first generation of mating will be obtained by intermating the p parents in all possible combinations. Thus, all members of the first generation will be F_1 individuals, and the first division will contribute s to the equivalent chromosome length rather than $\frac{s(p\text{-}1)}{p}$, if the parents were randomly intermated. For n meiotic divisions in the random mating population,

$$s' = s[1 + \sum_{i=2}^{n} a_i] = s[\frac{n(p\text{-}1)+1}{p}],$$

since the probability (a_i) in any generation $(n>1)$ of obtaining an effective break is $(p\text{-}1)/p$. The average segment length per chromosome can be calculated from (ix) for selected values of n, p, and s. As n becomes large the average segment length per chromosome approaches $p/[n(p\text{-}1)+1]$.

It is interesting that s' has an intuitive interpretation. If the effective breaks per chromosome are totaled over n meiotic divisions, the expected number of effective breaks per chromosome would be equivalent to a chromosome map length of $(s + s(p\text{-}1)/p + s(p\text{-}1)/p + \ldots)$.

For convenience the average segment length per chromosome after n meiotic divisions, or in the nth generation gametes from a population intermated as outlined, is given in Table 1 for selected numbers of parents (p), generation (n) and chromosome lengths (s). The lengths are expressed in the units of the genetic map scale. The chromosome lengths selected represent the range of chromosome lengths which might be found in an organism, based on available linkage measurements in corn (RHOADES 1955). Each parent used to synthesize the population contributes a progenitor homologue to the intermating population. With two parents $(p=2)$, segment lengths represent lengths of alternate linkage blocks from the two parents. With three parents, the segment lengths represent alternate linkage blocks involving units from three progenitor chromosomes.

The segment lengths in the first generation are identical for a selected s since all members are F_1 individuals. As noted by HANSON (1959b) the principal breakup of the longer chromosome linkage blocks occurs in the first 4–5 generations of intermating; however, the blocks are still of appreciable length at this stage of intermating. The effects of restricting the number of initial parents for the intermating population are not as drastic as one might suspect; however, the principal reduction in block length occurs in the first generation of mating. Since the first generation consisted of intercrossing of parents in all possible combinations, the full effects of restricting the number of initial parents are not manifested in the defined mating system. Intermating for four generations within a population synthesized from 3–4 parents is equivalent to at least five generations of intermating within a population from two initial parents with respect to reduction in average block length per chromosome. The arguments supporting the intermating of a population synthesized from a number of initial parents arise more from genetic ramifications than from reduction in block length.

TABLE 1

Expected segment length per chromosome (centimorgans/100) for selected chromosome map lengths (s) in the nth generation gametes of a random mating population where the first generation of intermating was obtained by intermating p homozygous parents in all possible combinations followed by random intermating

Chromosome length (s)	Number parents (p)	Generations of intermating (n)									
		1	2	3	4	5	6	8	10	16	30
0.5	2	.393	.352	.316	.285	.259	.236	.199	.170	.116	.064
	3	.393	.339	.295	.259	.229	.204	.166	.138	.090	.049
	4	.393	.333	.285	.247	.216	.191	.153	.126	.082	.044
	6	.393	.327	.276	.236	.204	.179	.142	.116	.074	.040
	10	.393	.323	.269	.228	.196	.170	.133	.109	.069	.037
	∞	.393	.316	.259	.216	.184	.158	.123	.099	.062	.033
1.0	2	.632	.518	.432	.367	.317	.277	.220	.181	.118	.064
	3	.632	.487	.387	.317	.266	.228	.176	.143	.091	.049
	4	.632	.472	.367	.296	.245	.209	.160	.129	.082	.044
	6	.632	.458	.349	.277	.228	.192	.146	.118	.074	.040
	10	.632	.448	.335	.264	.215	.181	.137	.110	.069	.037
	∞	.632	.432	.317	.245	.199	.166	.125	.100	.062	.033
2.0	2	.865	.634	.491	.397	.332	.286	.222	.182	.118	.064
	3	.865	.579	.424	.332	.272	.231	.176	.143	.091	.049
	4	.865	.554	.397	.307	.250	.210	.160	.129	.082	.044
	6	.865	.532	.373	.284	.231	.194	.146	.118	.074	.040
	10	.865	.514	.356	.269	.217	.182	.137	.110	.069	.037
	∞	.865	.491	.332	.250	.200	.167	.125	.100	.062	.033

The cumulative distribution for proportionate segment length per chromosome can be stated directly from the information presented by HANSON (1959b) and is

$$F(t_n) = 1\text{-}e^{-st_n[n(p-1)+1]/p}, \qquad 0 \leqslant t_n < 1.0.$$

Breakup of linkage blocks with random mating and selfing

The equivalent chromosome length as defined in (viii) for a selfed population is

$$s' = s + s/2 + s/4 + \cdots = s\left[\frac{2^n\text{-}1}{2^{n-1}}\right],$$

where n is the filial generation. As n becomes large, $s' \to 2s$. The average segment length per chromosome of progenitor linkage blocks intact at fixation can be summarized as follows, for selected chromosome lengths and segment lengths expressed on the proportionate scale and on the genetic map scale:

	Length of chromosome (s)			
Scale	0.3	0.5	1.0	2.0
Proportionate	.752	.632	.432	.245
Genetic	.226	.316	.432	.491

These figures, however, give an optimistic picture. Effective breaks result from recombinations which occur in heterozygous segments. Effective breaks which

occur in the $(n+v)$ generations occur within a region which is heterozygous in the nth generation of inbreeding. Effective breaks would occur at random along the chromosome in the meiotic division of the F_1 generation and could be considered approximately at random in the meiotic division of the F_2 generation. However, in subsequent generations, additional effective breaks would tend to occur in clusters within the chromosome map. For a chromosome of length 0.5, 79 percent of the chromosome is transmitted intact on the average in the meiotic division of an F_1 individual (Table 1, n=1). The additional reduction in block length to 63 percent through selfing represents additional breakup produced in part by localized recombination.

Consider a population synthesized by intermating p homozygous parents in all possible combinations. The $(n=1)$ generation would be a population of F_1 individuals. Randomly intermate the population for q generations, and then self within the population until fixation. The $(n=q+1)$ generation would represent the final generation of randomly intermated individuals, and $(n=q+2)$ generation would represent the first generation of selfing within the population. The equivalent chromosome length at complete homozygosity $(n=\infty)$ would be

$$s' = s[1 + q\ (p\text{-}1)/p + 2(p\text{-}1)/p] = s[p + (p\text{-}1)(q+2)]/p.$$

The average segment lengths per chromosome (centimorgans) of progenitor chromosome blocks intact in the selfing population at fixation are given in Table 2 for selected generations of random intermating (q), number of progenitor parents (p) and chromosome lengths (s).

TABLE 2

Expected segment length per chromosome (centimorgans/100) for selected chromosome map lengths (s) *for a population synthesized by intercrossing* p *homozygous parents in all possible combinations, intermating for* q *generations and selfing to complete homozygosity*

Chromosome length (s)	Number parents (p)	Generations of intermating (q)							
		0	1	2	3	4	5	7	9
0.5	2	.316	.285	.259	.236	.216	.199	.170	.148
	3	.316	.259	.229	.204	.184	.166	.139	.118
	4	.316	.247	.216	.191	.170	.153	.126	.107
	6	.316	.236	.204	.179	.158	.142	.116	.098
	10	.316	.228	.196	.170	.150	.133	.109	.091
1.0	2	.432	.367	.317	.277	.245	.220	.181	.154
	3	.432	.317	.266	.228	.199	.176	.143	.120
	4	.432	.296	.245	.209	.181	.160	.129	.108
	6	.432	.277	.228	.192	.166	.146	.118	.098
	10	.432	.264	.215	.181	.156	.137	.110	.092
2.0	2	.491	.398	.332	.286	.250	.222	.182	.154
	3	.491	.332	.272	.231	.200	.176	.158	.130
	4	.491	.307	.250	.210	.182	.160	.129	.108
	6	.491	.286	.231	.194	.167	.146	.118	.098
	10	.491	.270	.217	.182	.156	.137	.110	.092

The information in Table 1 can be used to help interpret the information available in Table 2. The average block length in the gametes from the $(n=q+1)$ generation represents the block lengths prior to the selfing generations. The additional reduction in block lengths following a selfing program is obtained by comparing the $(n=q+1)$ generations in Table 1 with the corresponding q generation in Table 2. The information from the two tables has been summarized for a few extreme cases in Table 3. The $(n=q+1)$ generation represents the average block lengths in the gametes from the final generation of intermating while $(n=\infty)$ represents the final average block length at fixation. Since effective breaks in the $(n+v)$ generations of selfing can occur only in segments heterozygous in the nth generation of selfing, the average block lengths in the gametes of the F_2 generation $(n=q+3)$ have been included for comparative purposes. The comparable generation with no random intercrossing would be $n=2$. Some question should be raised as to the comparability of the respective F_2 levels, specifically when p is small. The bias introduced by selecting the F_2 level favors specifically the $(p=2)$ case and would indicate a more favorable reduction in block length than that which actually occurred. However, the summary should serve for general comparative purposes.

Random intermating for four generations within a population synthesized from four parents is equivalent to about five generations of random mating with two parents with respect to the reduction of block length. Furthermore, with four

TABLE 3

Expected segment length per chromosome (centimorgans/100) for selected chromosome map lengths (s) *in the* n*th generation gametes of a population synthesized by intercrossing* p *homozygous parents in all possible combinations* (n=*1*), *intermating for* q *generations* (n=q+*1*) *and selfing to complete homozygosity*

Chromosome length (s)	Number parents (p)	Generation mating (n)	Generations of intermating (q)							
			0	1	2	3	4	5	7	9
0.5	2	$(q+1)$	.393	.352	.316	.285	.259	.236	.199	.170
		$(q+3)$	.352*	.300	.272	.247	.226	.207	.177	.153
		∞	.316	.285	.259	.236	.216	.199	.170	.148
	4	$(q+1)$	.393	.333	.285	.247	.216	.191	.153	.126
		$(q+3)$	.352*	.265	.244	.203	.180	.161	.132	.111
		∞	.316	.247	.216	.191	.170	.153	.126	.107
2.0	2	$(q+1)$	.865	.634	.491	.397	.332	.286	.222	.182
		$(q+3)$	.633*	.440	.362	.307	.266	.235	.190	.160
		∞	.491	.398	.332	.286	.250	.222	.182	.154
	4	$(q+1)$	.865	.554	.397	.307	.250	.210	.160	.129
		$(q+3)$	.633*	.347	.276	.228	.195	.170	.136	.113
		∞	.491	.307	.250	.210	.182	.160	.129	.108

* The generation is $n=2$, which is comparable to the second generation of selfing $(n=q+3)$ from an intermated population.

parents segments represent alternate linkage blocks with one of four types rather than two alternating progenitor segment types with two parents.

Since we are interested primarily in the equivalent breakup of linkage blocks under different breeding procedures, the segment lengths at the F_2 level ($n=q+3$) become a logical reference point for comparative purposes. It is extremely difficult to breakup linkage blocks for short chromosomes (0.5). Approximately, 30 percent (1-.352/.5) reduction in the original linkage block is achieved by selfing ($p=4$). The inclusion of one generation of intermating effects a 47 percent average reduction while the inclusion of four generations of intermating effects a 64 percent average reduction in the original linkage blocks. The corresponding block lengths with one and four generations of intermating included in the breeding system are about 3/4 and 1/2, respectively, of the average block lengths with no intermating. For the long chromosome ($s=2.0$ and $p=4$) the inclusion of one generation of intermating in the breeding system reduces the average length of segment by about 1/2 while four generations of intermating correspondingly reduce the average block length to 1/3, as compared to the average block length with no intermating.

DISCUSSION

The derivation of the average segment length per chromosome in the gametic array of a population subjected to a selected breeding system has been presented. The development was based primarily on theoretical considerations; however, any theoretical development requires experimental ramifications for support and justification. Unfortunately, experimental data from which the average segment length per chromosome can be estimated is not available and would be extremely difficult to obtain. The model must be critically examined in terms of the restrictions required for the development.

The assumption was made that the occurrence of chromosome breaks or points of genetic recombination is independent in a probability sense. The assumption is not restrictive since the number of crossovers occurring within a chromosome in any generation is limited and the breaks occurring in different generations are independent in a probability sense. The developmental procedure entailed the description of the distribution of chromosome types generated from n meiotic divisions. The probability that a chromosome will contain a total of x breaks or points of genetic recombination after n meiotic divisions is independent of breeding procedure; however, the probability that any break will involve a heterozygous segment depends upon the system of mating. An effective break is a break which involves a locus heterozygous for progenitor chromosome types. A limiting function was derived to define the distribution of chromosome types with respect to the number of effective breaks. Since the approximate distribution of chromosome types represents the limiting form, the feature is not restrictive as n becomes large. An intuitive concept of equivalent chromosome length was inherent in the limiting distribution. An equivalent chromosome length is the length of chromosome which would generate the distribution of effective breaks if all breaks were to occur in one meiotic division.

The concept of genetic map scale has arisen from the theory of genetic recombination involving simply inherited characters. It is entirely possible that the distribution of breaks is not uniform with respect to the physical length of the chromosome. The distribution would be uniform with respect to the genetic map scale. The assumption is made that the genes controlling quantitative characters are distributed in some manner within the genetic map scale. The failure to identify *s*, the length of a chromosome for a species, is not a restriction for the theoretical development but does represent a limitation in application. Information on the genetic map lengths for most species of economic importance is extremely limited. The measured map lengths in corn range from 0.28 for linkage group seven to 1.61 for linkage group one with an average of 0.91 (RHOADES 1955). These lengths would, of course, represent minimum lengths. The chromosome lengths of 0.5, 1.0 and 2.0 have been selected in this paper to represent the extreme ranges and the average for chromosome map lengths which might characterize a species.

The degree of homology between a pair of homologues will affect the length of the genetic map for a chromosome. Thus, a chromosome map length may be 1.0 for normal homologies; however, the reduction in homology between two homologous chromosomes, such as in an interspecies cross, would be equivalent to a reduction in chromosome map length to less than 1.0. With complete lack of homologous pairing, the chromosome map length would be zero. Thus, when a plant breeder is working with distant crosses, he may essentially be working with a material which is equivalent to a species with exceptionally short chromosome maps. When selection in a wide species cross involves a specific character, a backcrossing program would be followed; however, with the equivalence of a short chromosome map length the rate of breakup of the linkage block would be exceptionally slow (HANSON 1959c). Thus, a genetic map length for a chromosome is essentially a conceptial measure. Information available from a segment analysis will serve primarily as a point of reference.

It appears evident, based on the analysis of the breakup of linkage blocks, that a breeding program for a self-pollinated species should include at least one, and preferably three or four, generations of intermating if at all feasible. Genetic recombination within linkage groups is extremely limited, especially for short chromosomes unless intermating cycles are included in the breeding program. Furthermore, at least four parents should be used to synthesize the intermating population. The inclusion of four or more parents in the population increases the genetic potentials of the population and effects a greater reduction in average block lengths as compared to a population synthesized from two initial parents.

The inclusion of multiple sources of germplasm in the intermating cycle will require an extensive sampling of the intermating population to identify the desirable recombination. Selection for a simply inherited characteristic should be avoided in the intermating population since the selection for types would tend to fix chromosome segments in the intermating population and thus reduce the frequency of effective recombinations.

SUMMARY

The derivation of the average segment length per chromosome in the gametic array of a population under any breeding system has been presented. Segment lengths were taken with reference to the progenitor chromosome types for the population. Detailed considerations were given to a randomly intermated population synthesized from a limited number of initial parents and to a selfed population in which cycles of intermating have been included. The theoretical development has been presented in detail, and the ramifications for the restrictions imposed for the development were discussed.

The expected lengths of progenitor chromosome types intact in a chromosome after n meiotic divisions were detailed for the randomly intermating population and for the selfed population which included intermating cycles. The lengths were used to interpret the merits of selected breeding procedures.

If intermating in a self-pollinated species is at all possible, at least one or more, and preferably four, intermating cycles should precede the selfing generations to insure a degree of breakup of the linkage groups and to increase the genetic recombinations within the linkage group. At least four initial parents should be included in the intermating population. The inclusion of four or more parents in the population would increase the genetic potentials of the population and would effect a greater reduction in lengths of intact progenitor linkage blocks as compared to a population synthesized from two parents.

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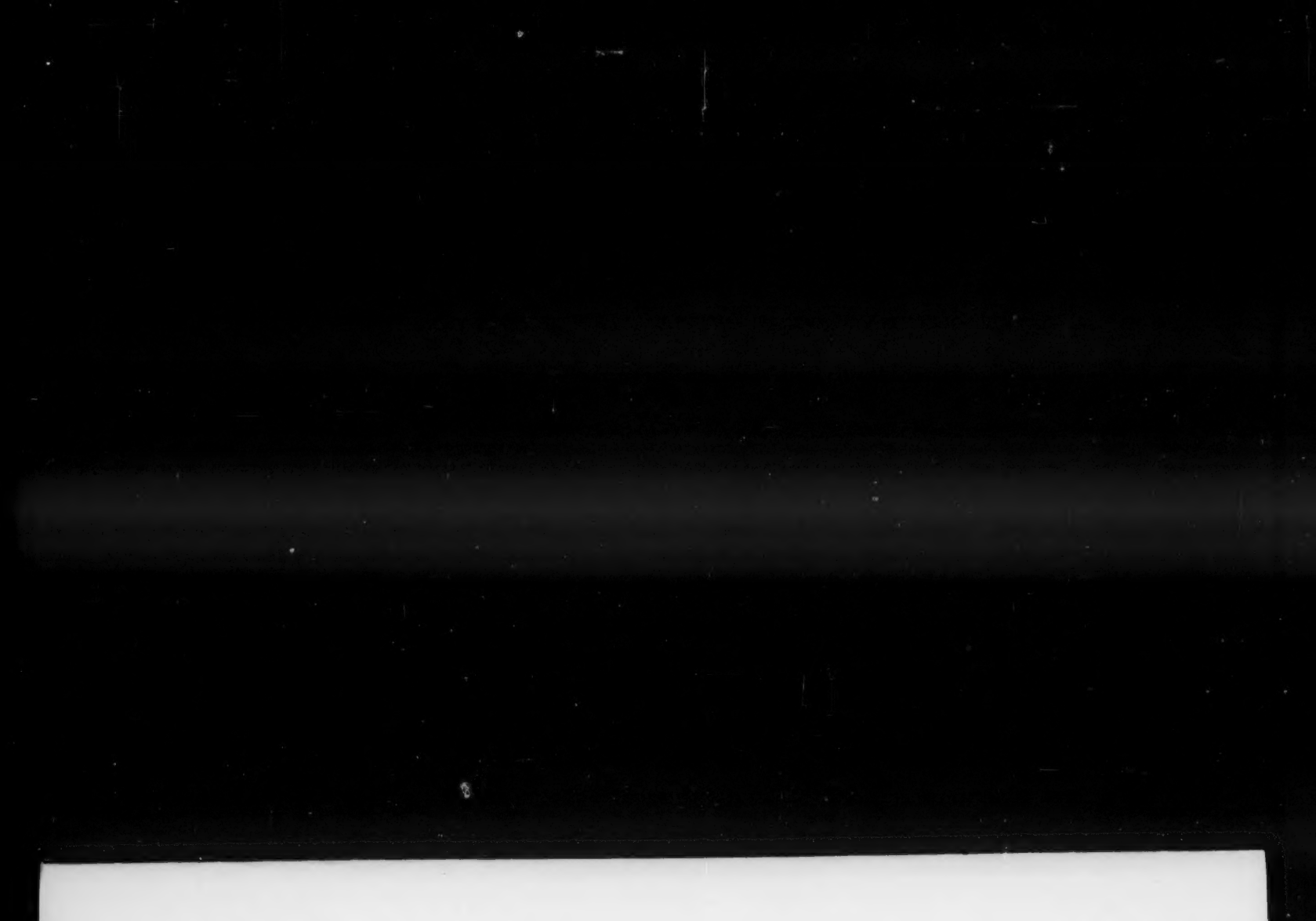
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